Vol. XIV. *February, 1908.* *No. 3*

BIOLOGICAL BULLETIN

THE ANNULUS OF A MEXICAN CRAYFISH.

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It is well known that the females of the common American crayfishes of the genus *Cambarus* possess a peculiar structure called in systematic descriptions the "annnlus ventralis." In our common northern crayfishes it has been shown (1) that the annulus contains an essential accessory reproductive organ, the seminal receptacle, without which the species would become extinct. Ortmann has recently divided the genus into six subgenera (2) and also (3 and 4) given reasons for supposing that the seventy or more species of *Cambarus* now living in the United States and adjacent territory are descended from forms once living in the region now called Mexico. As some of the more primitive subgenera are found only in Mexico and as it is not known whether the annulus in any Mexican crayfish contains a reproductive organ or not it seems worth while to describe the real character of the annulus in any Mexican crayfish that can be obtained.

The question as to the evolution and spread of the genus *Cambarus* cannot be completely answered till it is shown that the annulus in all members of the genus does contain the sperm receptacle, and that they thus differ from all other crayfish the world over.

The crayfishes whose annulus is here described were bought in the market of the city of Mexico, in July [1] and prove to be *Cambarus montezumæ* Saus. of the typical form.

This species was first described in 1857 by Saussure (5), but

[1] These specimens were obtained by Horace Andrews, C.E., with the aid of W. W. Blake. They were on sale, cooked, as taken in Lake Zumpango and known by the Astec name "Acociles."

without mention of the annulus; subsequently Martens (6) described a variety and Faxon (7) gave a new account of the typical form, of which he says the annulus "is movable, fixed only at the posterior end, between the sterna of the penultimate and last thoracic somites. The ventral face of the annulus is marked by a longitudinal fossa open at the posterior end" (page 122). Several varieties of the species were later briefly described by the same author (8). Recently Ortmann has examined the specimens of Mexican crayfishes from the Paris Museum (2) and mentioned in a footnote that the peculiar spine posterior to the annulus in his new subgenus *Paracambarus* distinguishes this from all other Cambari except *C. montezumæ*.

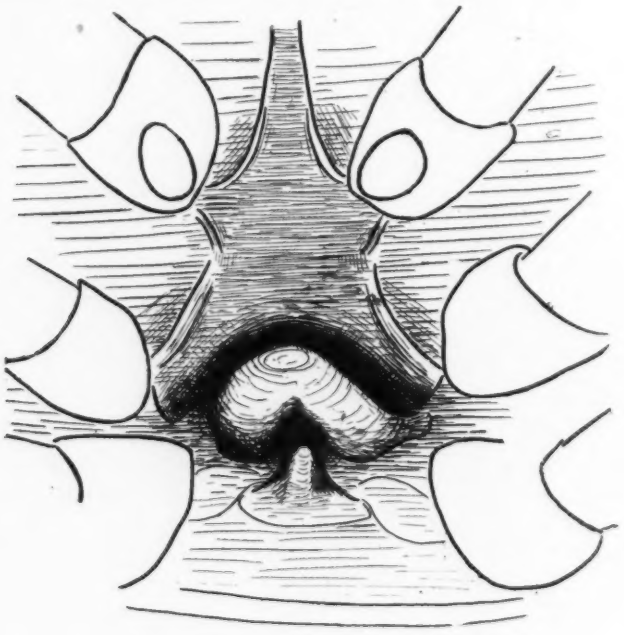

FIG. 1.

The above is all thus far known regarding the annulus in *Cambarus montezumæ*, or in the subgenus *Cambarellus*, in which it is placed by Ortmann.

Of the 179 specimens sent to me 88 were females ranging in length from 16 mm. to 36 mm., many being 25–28 mm. The males ranged from 14 to 36 mm. and many were 25 mm. Saussure had 25 specimens, which measured up to 27 mm., while Faxon's largest was 35 mm. That those 30–36 mm. were sexually mature was evident from the fact that six bore eggs, and two, young larvæ, attached to the abdominal appendages, as in other crayfishes.

The general appearance of the annulus in these small, but mature forms is indicated in Fig. 1, which shows the bases of the third to the fifth thoracic legs and the sternal surface between them. While the annulus has the same position as in all other Cambari, between the sterna of the fourth and fifth legs, it differs from any hitherto figured in not being a low transverse plate of more or less annular form but in forming a high transverse papilla of asymmetrical form.

The sternum between the fifth legs is also more specialized than in higher Cambari in that it projects as a stiff spine, somewhat like a lengthwise ridge, but often more like a conical spine.

This spine fits into a very marked median groove on the posterior side of the annulus in the manner mentioned by Ortmann (2) as characteristic of his new form, *Paracambarus paradoxus*. The eggs issuing from the elliptical openings on the third legs (Fig. 1) doubtless flow back around the base of this rounded, papilla-like annulus, and there receive the fertilizing sperm, for we find in this annulus a functioning sperm receptacle.

The appearance of the annulus when cut off and viewed from the posterior side is shown in Fig. 2. The right and left of the high papilla are unevenly balanced about the long deep median groove and on the observer's left, which is the animal's right, there is a peculiar structure, which proves to be the seminal receptacle. The asymmetry of the annulus is marked, the apex being to one side and the side containing the receptacle being abrupt while the opposite one slopes gradually.

In the crayfish hitherto studied the receptacle is wholly, or in part, on the median line but here we find it entirely to one side. In those crayfish there is, as far as studied, a peculiar dimorphism amongst the females (9), some of them having the entrance of the

receptacle on the right side of the body and some on the left. In this Mexican crayfish it is surprising to find that the dimorphism

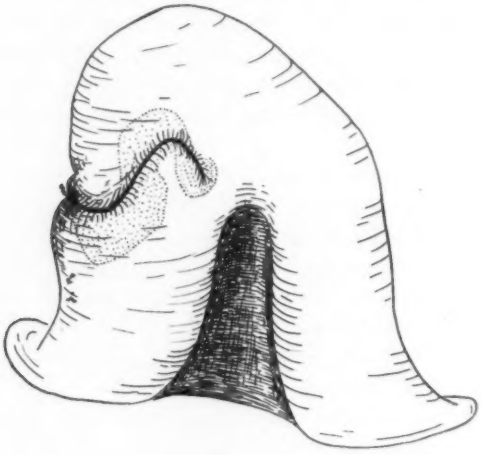

FIG. 2.

is expressed by a transposed position of the entire receptacle. As it happened just forty-four of the females had the receptacle on

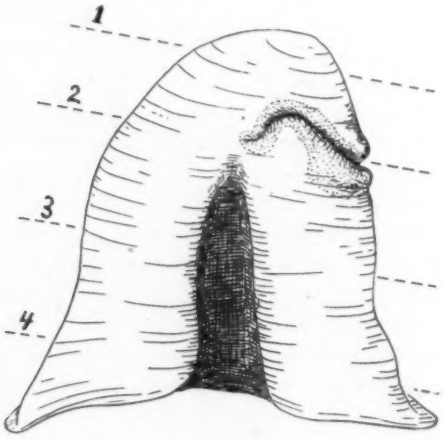

FIG. 3.

the right as in Fig. 2 and the other forty-four on the left. In these left-handed females the posterior side of the annulus appeared as in Fig. 3. Leaving out of account the differences in height and width and the altitude of the receptacle, which are diverse in individuals either dextral or sinistral, the annulus in Fig. 3 is the mirror image of that in Fig. 2: the abrupt and the slop-

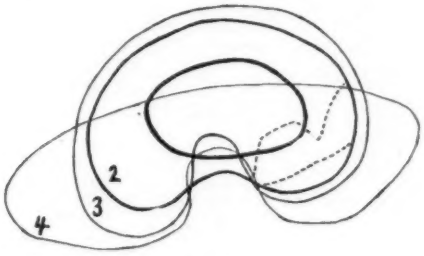

FIG. 4.

ing sides and the position of the apex are reversed and this is obviously associated with the fact that the receptacle is on opposite sides in the two cases.

The real shape of the annulus in either sinistral or dextral forms is seen only after observation from different points of view. The annulus is wide from side to side and compressed from before back, very protuberant in front and less so behind and has a very small base, which accounts for the movability noticed by Faxon. A view of the sinistral annulus (Fig. 3) taken from the side that bears the receptacle is shown in Fig. 5, which emphasizes the very narrow base and the greatly protruding anterior face. The apex is also seen to rapidly taper. The receptacle obviously extends around onto this lateral side above the level of the central groove, which is indicated in broken lines as seen through the side. Upon looking down upon this same sinistral annulus

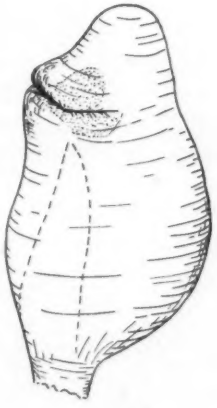

FIG. 5.

from above, successive optical sections presented the outlines seen in Fig. 4: the heavy black line is the outline of the apex above the receptacle; the line No. 2 is the circumference of the annulus around the level (2) of Fig. 3, and in the receptacle is represented by dotted lines; the line 3 shows the maximum thickness from before back, also the greatest depth of the median groove; finally the line 4, Figs. 3 and 4, shows the great width of the annulus and its compression from before back, near its base. An anterior view of this same annulus was like Fig. 3 but with the sides reversed and with the annulus and median groove invisible until the annulus was made transparent, when they were seen through its substance.

By study of annuli cleared whole and also cut into sections the internal structure was seen to agree with what was made out for other annuli (9). Thus a lengthwise section (Fig. 8), shows a thick exoskeleton lined by nucleated epidermis and a central mass of areolar connective tissue full of blood sinuses and vessels with no discovered musculature. The shell on the straighter posterior face seems thicker than on the more protuberant anterior face. The infolding near the upper end was the part of the receptacle of this dextral annulus, cut near the right face.

As a protruding mass of connective tissue covered with thick exoskeleton the annulus in other crayfishes forms a stiff plate more or less movable since the exoskeleton round about it is less thick and more pliable, but in *Cambarus montezumæ* the mobility is apparently enhanced from the fact that the great height of the mass and its narrowed base make it easy to rock it back and forth. The convex front face of the annulus fits against the hollowed out sternal plate of the somite bearing the fourth legs, as s imperfectly indicated in Fig. 1.

As the spine on the middle of the sternum behind the annulus is on that somite of the thorax which can be independently moved by the animal, it may be that the annulus is sometimes caught between the spine that enters its posterior groove and the solid sterna in front of it and subjected to pressure and this may be a means of emptying the receptacle.

The receptacle itself proves to be made precisely along the same lines as in the other crayfish in which it has been described

(9): it is a rather simple infolding of the epidermis, lined by the exoskeleton and is a mere flat pocket opening to the exterior by a narrow chink.

The mouth of the sperm pocket is the sinuous line seen in Figs. 1, 2, 3 and 5, passing as an S-shaped line from the neighborhood of the median groove to the right or the left as the case may be, across the posterior face and then around onto the lateral face of the annulus. It is not extended onto the anterior face. For most of its extent the line is a closed suture, or mere morphological mouth, forming a chink that is apparently closed up, but toward the edge of the posterior face and on the lateral face the chink is more patently an opening into the interior. The lips of this suture are more or less swollen especially on the lateral face and toward the edge of the posterior face and this is much more pronounced in some individuals than in others (Figs. 2 and 3).

The internal pocket into which the suture leads may be seen through the exoskeleton when properly prepared and, as indicated in the above figures by the dotted areas, it extends out rather far on either side of the suture in a peculiar way.

A good idea of the shape of the internal pocket may be got by looking at it from the inside, as in Fig. 6, that is, by removing the anterior walls of the annulus and all the connective tissue and epidermis; the exoskeleton that lines the sperm pocket stands forth as a prominent ridge passing sinuously from side to side. Fig. 6 is such an anterior view of the exoskeleton of the sperm-pocket of a right-handed annulus, similar to Fig. 2. The flat, sinuous ridge there indicated is attached all along the posterior side of the annulus and free anteriorly, toward the observer; the part to the right is the beginning of the pocket on the lateral face of the annulus and stands forward as do the middle and the left parts, forming three roughly parallel parts of an S.

FIG. 6.

The extreme tip to the left, as well as two intermediate regions, are lower and are bent alternately down, up and down. Thus one sees on the right only the top of the ridge, then part of its upper or distal surface, then the top, then part of its lower or proximal surface, then the top again and finally the upper surface at the end. By this mode of bending as a warped surface the edge of the pocket, or ridge, toward the observer is longer than the anterior edge, which is the mouth, so that the same condition is found as in higher crayfish and the sperm pocket is like an elastic coat pocket which should have its mouth bent in an S and its bottom pulled out into an S with longer loops, after the fashion of a mesentery that has a shorter origin and a longer line of attachment. Seen on the outside (Figs. 2 and 3) this greater bending of the internal than of the external

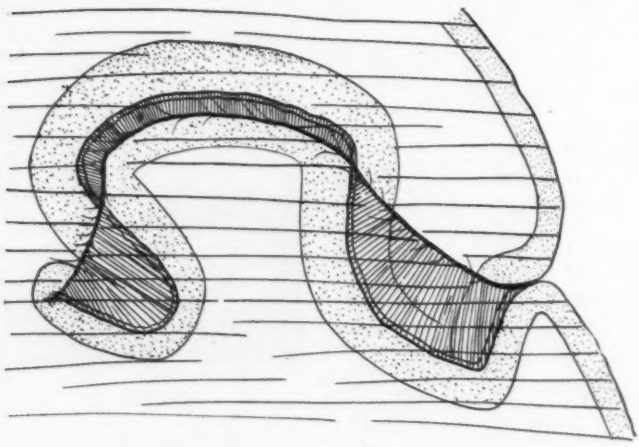

FIG. 7.

limiting edge is evident and variable. In old well-marked annuli the pocket (Fig. 2) drops down from its mouth in a long sweep toward the base of the annulus and then sweeps up above the suture toward the summit to return again toward the median end of the suture. In other cases (Fig. 3) the pocket is less drawn out toward the summit and stands more at right angles to the surface. When more magnified such a receptacle seen from the

outside as a translucent object is represented in Fig. 7. The line of the mouth coming around from the left face makes an imperfect S which is much shorter than the inner edge of the pocket walls, indicated as a continuation of the outside shell. Through the thick exoskeleton runs only a narrow crevice the outer mouth of which is shown as the black line or suture and the bottom of which is indicated by two parallel lines which cross the suture at two points. The sides of the chink, or cavity of the pocket, are diagrammatically represented by ruled lines. The cavity of the pocket thus runs in from the mouth at first obliquely downward and to the observer's left then slightly upward and then downward again and toward the observer's right.

In other cases (Fig. 10) the middle loop toward the apex of the annulus is much more pronounced. The simple nature of

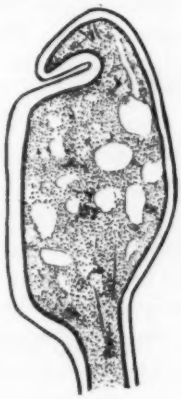

FIG. 8.

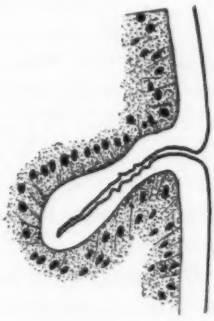

FIG. 9.

the pocket is shown in the lengthwise section of the annulus (Fig. 8) which shows the narrow cavity of the pocket of a dextral annulus near its origin toward the right face of the annulus.

Other sections show the same simple pocket, whenever cut at right angles to its twisting course. In Fig. 9 is shown, enlarged, the terminal part of a dextral pocket cut near its median termination where it slopes away from the surface toward the median plain and also down toward the base of the annulus. Here as elsewhere the pocket is seen to be really an invagination of the

epidermis lined with exoskeleton that has the same shape. By careful dissection this epidermis was removed in other cases as a hollow mould in which the exoskeleton had fitted.

That this thin, flat slit in the shell of *Cambarus montezumæ* actually is used as a sperm reservoir is well proven on two specimens that show the sperm as it was issuing out of the mouth of the pocket. One of these (Fig. 10) was a dextral annulus like Fig. 2. In this figure the bottom of the pocket is represented by dotted parallel lines and the exoskeleton by parallel rulings. The outer mouth or suture is shown as a black line. Extending out from this on its middle loop is a large area covered with dots and these were round, flat, clear bodies which with 6 and 2 mm. objective clearly showed the characteristic central bowl of crayfish spermatozoa. These sperms were issuing out of the middle loop of the suture and spreading on either side over the surface of the annulus where they might meet the eggs (Fig. 1). The sperm seemed to be in a single layer over a considerable area, though in a deeper mass where emerging from the suture. They lay flat side by side and their clear outer part around the central bowl suggested that they might be gliding along somewhat like a liquid wetting the annulus. No radiating arms were made out, so that here again the male must have succeeded in transferring the sperm into the annulus without the expansion of the sperms into those stars which were so often produced by the liquids used by investigators as to be regarded as the only shape of the mature sperm. The part played by the male must be the same as in other Cambari for an examination of the first male pleopods revealed some of these same round sperm cells issuing out of the tip of the "canula" or discharging tip of that appendage, which is doubtless inserted into the receptacle.

Whether the above figured sperms were forced out at the time the animals were killed, or just before, is not known, but the appearances are that pressure of some kind must be applied not only to get the sperm into such a stiff rigid pocket (Fig. 9) but also to get it out again. In another specimen the sperm had issued out of the suture all along its median as well as its middle loop and this was probably the case in Fig. 10 before it was examined.

In both cases the first loop of the suture showed the thin plate of wax-like material represented in Fig. 10 as the darkened mass along the first curve of the suture. This wax also projected on the lateral face as the "sperm plug" so much more conspicuous in *C. affinis*. Now the oozing out of such a film of wax and the extension of the sperm from the suture over the annulus is what

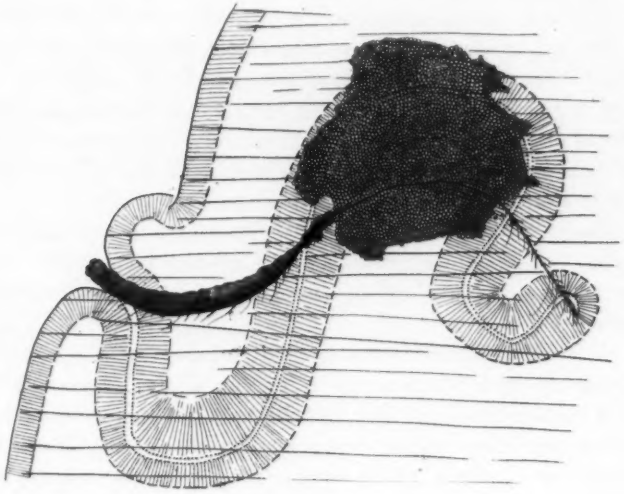

FIG. 10.

was made out, with difficulty, in the specialized annulus of *C. affinis* when mechanical pressure was exerted upon the annulus by forcing the hard sternum of the last thoracic somite against the annulus.

In these *C. montezumæ* the sperm may have been forced out of the pocket by the shrinkage due to the heat with which they were probably killed, or the issuance of sperm may have been a normally produced act in females about to lay and in that case the efficiency of the spine upon the sternum of the fifth legs (Fig. 1) as a means of squeezing the annulus against the sterna in front of it, seems to support the view that it is such spontaneous pressure on the part of the female that liberates the sperm at the right time to meet the eggs.

Comparing the annulus of *Cambarus montezumæ* with that of other Cambari we see that though it departs so much in general form yet it agrees in containing a sperm pocket that is both in structure and use fundamentally identical with the sperm pockets of other Cambari. In all cases known the male fills the inner curves of the pocket with sperm and the outermost curve, near the end into which the male pleopod is thrust, with a wax or cement that seals the sperm in.

The sperm pocket here described is strikingly like that previously described for *C. immunis* (9), both in the simplicity and form of its bendings and in the fact that it runs transversely instead of longitudinally as in most crayfish. Again the form of the pocket here described agrees closely with that of *C. clarkii* (9) though the latter is a longitudinal, median pocket.

The sperm pocket of *C. montezumæ* thus resembles the simplest sperm pockets known, those most like an early stage of the more specialized pocket of *C. affinis*, whose ontogeny has been described elsewhere (10).

As far as the sperm pocket is of any value in indicating phylogenetic affinity it points to the conclusion that *C. montezumæ* is not a highly specialized form, but need not be taken to mean that there is any close relation between *C. montezumæ* and either *C. immunis* or *C. clarkii*, which other characters indicate are remote. And the value of similarity in form of the sperm pockets in these three is nullified by their different positions on the annulus. The ontogeny of the sperm pocket in *C. affinis* shows that it starts as a median groove which secondarily has added to it the part right or left of the median line and if this is the general rule for other Cambari such wide departure from the median position as that in *C. immunis* and *C. montezumæ* may well mean remote connection with such a median form as that in *C. clarkii* and if the ontogeny of the annulus in *C. montezumæ* were known the close resemblance of its sperm pocket to that of *C. immunis* might prove to be a superficial one.

As yet of the six subgenera into which *Cambarus* has been divided by Ortmann the sperm pocket has been made known in four: in *C. affinis*, *C. virilis*, *C. immunis*, representing the subgenus *Faxonius;* in *C. bartoni*, representing the subgenus *Bar-*

tonius; in *C. clarkii*, representing the subgenus *Cambarus* and in *C. montezumæ*, representing the subgenus *Cambarellus*. It would thus be premature to use the facts known as to the sperm receptacle as a guide where they may clash with other criteria for determining the history of the group.

Observations upon the sperm pockets of the two other subgenera, *Paracambarus* and *Procambarus*, both well represented in Mexico, are to be desired.

BALTIMORE, December 1, 1907.

EXPLANATION OF FIGURES.

Figures are all drawn with camera lucida and the Zeiss lenses indicated: Fig. 1 with 2.90 mm.; Figs. 2, 3, 4, 5, 6, 8 with 2A.; Figs. 7, 9, 10 with 2D.; and reduced to ⅓ diameter in Fig. 10 and ½ in the others.

REFERENCES.

1. **Andrews, E. A.**
'97 Amer. Nat., 29, 1897.
2. **Ortmann, A. E.**
'06 Proc. Wash. Acad. Sci., VIII., 1906.
3. '02 Proc. Amer. Phil. Soc., 41, 1902.
4. '05 Proc. Amer. Phil. Soc., 44, 1905.
5. **Saussure.**
'57 Rev. et Mag. de Zool., 2d ser., IX., 1857.
6. **Martens.**
'72 Archiv f. naturgesch., XXXVIII., I., 130, 1872.
7. **Faxon.**
'85 Mem. Harvard Mus., 1885.
8. '99 Proc. U. S. Nat. Mus., XX., 1899.
9. **Andrews, E. A.**
'06 Proc. Boston Soc. Nat. Hist., 32, 1906.
10. '06 Biol. Bull., 10, 1906.

THE ADHESIVE ORGANS OF AMIA.

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Most of the ganoid fishes have adhesive organs or sucking disks in the early stages of their development.

These organs have received little attention even from morphologists and systematists. Their development has not been worked out with any completeness in any species, but enough has been learned to raise numerous questions. The earliest stages in their development have never been described; even the germ layer from which they take their origin is unsettled. Their relations to other systems, digestive system, nervous system, etc., are in dispute. The various stages in their development, their structure during these stages and the functions they perform, as well as questions of their ultimate fate and their phylogeny and meaning seem to us to merit further study.

In a previous paper ('06) on the "Development of Amia" we have touched briefly on these organs. The present paper is a continuation of the same subject and is based on the same material.

Description of Stages.

The anlagen of the adhesive organs appear in the embryo about seventy hours after fertilization, or more precisely, when the embryo extends over about 160 degrees of the circumference of the egg. A median sagittal section of the anterior portion of an embryo of this stage is shown in Fig. 1. The ectoderm consists of a single layer of cells externally (*s.ec.*) and a large mass of deeper lying cells; the deeper layer is some 15 to 20 cells in thickness. In this mass of cells which forms the anlage of the future brain there is a slight cavity (*br.c.*) which is the first cavity to appear in the brain. In front of the brain is a second mass of thickened ectoderm (*p.cb.*) which has been called the precerebral mass. The gut-cavity (*g.c.*) is now widely extended laterally. In its anterior portion its dorsal and ventral walls are

closely apposed, yet they can be traced as distinct layers to a point immediately below the pre-cerebral mass. It is in this locality that the adhesive organs appear.

In an embryo of ninety hours which covers about 180 degrees of the circumference of the egg the adhesive organs are not observable in surface views but in sections their beginnings are obvious. Fig. 2 represents an obliquely sagittal section of the anterior portion of an embryo in this stage. The superficial ectoderm (*s.ec.*) consists of two layers of cells which are invaginated just at the anterior boundary of the forebrain (*f.b.*). The brain cavities are now well defined. There is, however, as yet no indication of the infundibular or epiphysial evaginations. The notochord extends nearly to the level of the anterior end of the brain, but owing to the obliquity of the section its anterior portion is not represented in the figure. The cavity of the gut is well defined beneath the posterior portion of the brain; it is greatly reduced in size anteriorly but after reaching the level of the ectodermic invagination described above, it again expands into a wide cavity (*g.d.*). The walls of the gut show little change until the head region is passed when the cells of the dorsal wall change from flat or cuboidal to columnar. These greatly thickened areas of the entoderm form the beginnings of the adhesive organs.

Transverse sections of this stage (Fig. 3) show that the organs have arisen as paired diverticula of the anterior end of the foregut.

In an embryo of one hundred and twenty-five hours, covering about 260 degrees of the circumference of the egg the adhesive organs show plainly in surface views as paired structures lying on either side of the median line. An obliquely sagittal section passing through one of the organs is represented in Fig. 4. It will be noted that the lumen of the brain is enlarged and its subdivisions more clearly marked. The dorsal wall of the forebrain now shows a slight evagination which is the beginning of the epiphysis; just opposite in the floor is another evagination which is the beginning of the infundibulum. The foregut is here well shown with its forward extension into the pre-cerebral region where it ends in a wide dilatation (*g.d.*). The dorsal wall of this cavity is composed of elongated entodermal cells essentially similar to those seen in Fig. 2. Just beneath the adhesive organ is one of

the lateral evaginations of the cœlom which later unites with a corresponding structure on the opposite side to form the heart.

Fig. 5 shows an obliquely transverse section through an embryo somewhat older. The anterior end of the forebrain (*f.b.*) appears as a solid mass of elongated cells. In connection with its ventral wall, the optic stalk passes obliquely outward and terminates in the optic vesicle (*o.v.*). On the other side the section passes through the anterior portion of the adhesive organ (*a.o.*) which here shows its connection with the anterior end of the foregut (*g.*). The foregut is almost closed off ventrally through the approximation of the cœlomic cavities. Between the end of the brain and the optic vesicles there is a slight invagination (*n.*) of the deep ectoderm to form the beginning of the nasal pit. The character of the epithelium of the adhesive organ is now more plainly shown owing to the absorption of yolk granules. It will be noted that the adhesive organ is now in contact with the superficial ectoderm (*s.ec.*) which extends over it as a double layer.

Another section through an embryo a few hours older taken in a sagittal plane is shown in Fig. 6. The section passes through the embryo in such a direction that one of the adhesive organs is cut lengthwise. The organ at this time is still in open connection with the gut. Its antero-dorsal surface follows closely the external contour of the embryo. The peripheral end of the organ thus extends both posteriorly and mesially following the course of least resistance. In many of the sections of this stage, or slightly later, a constriction is formed around the middle of each of the large organs which soon gives rise to a pair on each side of the snout.

The minute structure of the organ is very similar to that shown in Fig. 5. It is composed of very high columnar epithelium that presents a pseudo-stratified appearance. These columnar cells are heavily laden with yolk granules which are very large and dense at the bases of the cells but gradually diminish in density and size toward the inner ends of the cells until these ends become free from yolk. These yolk-free ends show that cellular metabolism is here most active. The indications are, from the condition of the reticulum and the stainable fine granules, that they are secreting cells. Moreover the presence of small droplets on the surface of the cells adds confirmation to this view.

The section of the organ represented in Fig. 7 is from a larva in which the head and tail are both free from the yolk.

The organs are now entirely detached from the gut although the two layers of the entoderm forming the gut diverticula are still discernible. They lie, for the most part, in the ectoderm. They have forced their way through the deeper layers and are covered externally by only a single layer. Even this layer is now very thin and the cell boundaries are no longer well defined. The two organs are now separated from each other and each is subdivided into six to ten separate disks. Fig. 7 represents a section through a single disk. The minute structure of the organ is similar to that previously described. It is composed of pseudo-columnar cells in which the nuclei are situated nearer the bases of the cells than hitherto noted. The basal half of each cell contains some large yolk granules and a coarse reticulum which takes a deep hæmatoxylin stain. The peripheral half of the cells, on the other hand, is free from yolk but contains a fine reticulum which stains only faintly with hæmatoxylin. This faintly staining portion of the cell contains minute granules which are best interpreted as prozymogen granules. Although the organ has all the appearances in the present stage, as it had in several of the preceding stages, of a mucous secreting structure it is not yet functional as an adhesive organ. One remarkable change has taken place in the structure of the organ. The entodermal cells show a changed polarity. In the preceding stages the most active cell metabolism was in the ends of the cells next to the gut cavity ; now the most active cell metabolism is in the outer ends of the cells next to the exterior of the body, while the earlier clearer ends are now filled with fine granules.

In the larva of 4–5 mm. the organs have broken through the superficial ectoderm and open directly on the surface of the body, although they are as yet partly covered by the ectoderm, as a glance at Fig. 8 will show. The cells of the organ here shown are not different from those described in the preceding stage excepting that the clear zone of the cell appears to extend over one third of its length instead of one half.

In the larva of 8–9 mm. (Fig. 9) the disk has changed from an oval to a rectangular form as a comparison of Figs. 8 and 9

will show. The ectoderm (*s. ec.*) has undergone considerable thickening and differentiation. It no longer extends over any portion of the organ and as a result a far greater number of cells now reach the surface. The character of the cells is somewhat different as a result of the general change in the form of the organ. They are now typical, pseudo-stratified columnar cells while in preceding stages the outer ends were compressed. The cells no longer possess yolk granules while the prozymogen granules show more plainly in the outer ends. That some sort of a mucous secretion is furnished by these cells is indicated, not only by the cellular structure but also by the fact that when the larvæ are detached from the various objects, to which they now adhere, it is often observed that fragments of aquatic plants cling to the organs.

The larva of 13–14 mm. begins to move freely from place to place. It rarely attempts to attach itself as in the preceding stages. Corresponding with this change in the behavior of the young fish there is a marked change in the position of the adhesive organs. When viewed from the surface, some of the disks forming the horseshoe are still distinctly visible while others are barely discernible. All, however, show a great reduction in number when compared with the larva of 8–9 mm. A section of one of these organs is shown in Fig. 10. It will be noted that the entire organ has now sunk below the level of the epidermis and is partly surrounded by dermal pigment. The organ is still in communication with the exterior through a funnel-shaped opening in the epidermis.

In the larva of 18–20 mm. the organs have sunk still deeper below the level of the epidermis. Some of them still communicate with the exterior, while others are completely covered by the epidermis. Those that communicate with the exterior show a long narrow epithelial tube with numerous branches or diverticula at its inner end. The walls of these tubes are several layers of cells in thickness as shown in Fig. 11. Toward the inner end of the tube the epithelium becomes thinner until there remains but a single layer of cells covering the smaller branches. This layer becomes broken up in the smallest branches and these epithelial cells become swollen, the nuclei show chromato-

lysis and the cells are absorbed. The ectodermal cells forming the organs, as shown in the figure, likewise are much swollen, their outlines are indistinct and connective tissue grows in among them. Their nuclei are less readily stained with basic stains. The cytoplasm becomes vacuolated and granular; all of which points to albuminoid degeneration.

Historical and Critical.

The first author to treat of the development of the adhesive organ of any ganoid was Alexander Agassiz ('78) in his paper on *Lepidosteus*. But his observations were limited to the period after hatching. At this time the development of the organ is nearly completed and his descriptions therefore refer mainly to its function and its degeneration and disappearance. He describes the "huge mouth cavity" of the newly hatched *Lepidosteus* "surmounted by a hoof-shaped depression edged with a row of protuberances acting as suckers" and compares it with the mouth of the cyclostomes. The moment the little fish is hatched it attaches itself firmly to the side of the dish and there remains hanging immovable. Three days after hatching the disk becomes more prominent, "the individual suckers projecting frequently beyond the general outline of the edge of the suckers." As the snout lengthens the suckers become concentrated and the size of the terminal disk is reduced. When the fish is three weeks old "the sucking snout is now reduced to a swelling of the extremity of the elongated upper jaw." Later the disk is reduced to a single row of small suckers and finally it becomes the fleshy globular termination of the upper jaw of the adult.

Balfour and Parker ('82) continued the work on *Lepidosteus*, working on material furnished by Agassiz. They state that the disk is formed two or three days before hatching but give no details. They give two figures showing something of its histological structure and add: "The result of our examination has been to show that the disk is provided with a series of papillae often exhibiting a bilateral arrangement. The papillae are mainly constituted of highly modified cells of the mucous [inner] layer of the epidermis [epiblast?]. These cells have the form of elongated columns, the nucleus being placed at the base and the main

mass of the cells being filled with a protoplasmic reticulum. They may probably be regarded as modified mucous cells." In regard to its function they say: "It does not appear probable that the disc has a true sucking action. It is unprovided with muscular elements, and there appears to be no mechanism by which it could act as a sucking organ. We must suppose, therefore, that its adhesive power depends upon the capacity of the cells composing its papillae to pour out a sticky secretion."

Von Kupfer ('93) has described the development of the head in *Acipenser* basing his studies on median sagittal sections. He derives the adhesive organ from the inner layer of the ectoderm and states that it arises in close connection with the hypophysis. It is at first single but becomes paired by a groove on the median line. Each of these disks divides again, forming four papillæ. These four papillæ develop into the four barbels of the adult fish.

In regard to his derivation of the adhesive organs from the ectoderm we may say: (1) Kupfer's work is based on median sagittal sections and such sections are not those best adapted to trace out the development of a paired organ like the adhesive organs. (2) He has not described the earliest stages. His youngest stage is an embryo forty-five hours after fertilization. (3) Even by Kupfer's showing these organs are connected with the digestive system, though it may be only through the hypophysis.

Ehrenbaum ('94), as stated by Ziegler ('02, p. 157), gives a different origin for the adhesive organs in *Acipenser*. We translate from Ziegler: "On the under side of the head in front of the mouth one finds on each side two thickenings (Wülste) which become the barbels. At this place we noticed in a somewhat earlier stage a depression marked by a peculiar pigmentation; this corresponds to the sucking disk of *Lepidosteus* and *Amia*." How this is to be reconciled with von Kupfer's account of a common anlage for adhesive organs and hypophysis is not apparent to us. Unfortunately Ehrenbaum's paper is not accessible and we are not in position to give any further account of his work.

On the whole then, while we have the high authority of von Kupfer in favor of the view that in *Acipenser* the adhesive organs take their origin from the ectoderm, in our opinion the subject deserves further investigation as the proof is far from being conclusive.

Dean ('96) was the first author to treat of the development of the adhesive organs in *Amia*. His description begins with an embryo 138 hours after fertilization, that is, about two days before hatching. At this time the organ is already well defined; he notes its paired character and states that at hatching it is relatively at its largest size. Most of his observations have reference to its degeneration and disappearance. This process begins in a few days after hatching. By the seventh day the organ "is reduced to a mere tubercle and is no longer functional." On the tenth day "it is greatly reduced, although it does not in fact disappear entirely (histologically) for several weeks. . . . Its atrophy takes place first proximally, later marginally; the cells of its deepest tissues become greatly vacuolated and form a sponge-like mass and the cell wall which here forms its anterior boundary gradually encroaches; the cells of the centro-distal region are the last to retain their early character."

In his general discussion of his observations he homologizes the adhesive organs with "the typical pit organs, or sense buds which later occur on other integumental regions" and uses this homology as "evidence of how precociously embryonic and larval structures may be developed." Needless to say we find no basis for any such homology and no grounds for mentioning the subject of precocity.

In 1899 Miss Jessie Phelps working in Reighard's laboratory published a brief article on the "Development of the Adhesive Organs of *Amia*." This writer says: "The organ is formed in a very early stage as a diverticulum of the foregut. This diverticulum subsequently divides into two, each of which continues to communicate for a time with the cavity of the foregut. Each of the two diverticula later separates from the foregut, becomes elongated and curved into the form of a semicircle and divides into from six to eight closed vesicles. The vesicles finally open to the exterior and are thus converted into cups. After being functionally active for a time the organ is pushed beneath the surface by the thickening ectoderm, becomes infiltrated with leucocytes and finally disappears (larvæ of 20–25 mm.) without leaving any trace behind it."

The results of our work confirm the findings of Miss Phelps a

to the germ layer from which these organs are formed. But we cannot agree with her statement that the organ consists at first of a single diverticulum which subsequently divides into two. We find the anlagen of the adhesive organs in paired thickenings of the dorso-lateral portion of the anterior extremity of the foregut (Fig. 3). As soon as these become recognizable as diverticula there are plainly two of them.

As to their method of disappearance we are unable to find anything which indicates an infiltration of leucocytes or phagocytosis. On the other hand everything suggests an autogenous degeneration.

It has been shown beyond question that in *Amia* the adhesive organs are developed from the entoderm. But in the other ganoids, *Acipenser* and *Lepidosteus* — while the proof is not conclusive — the balance of authority favors the view that they are formed from the ectoderm. *A priori* it does not seem probable that homologous organs would be formed from different germ layers. But what proof have we that these organs are homologous? Sucking disks are formed in many species of animals in widely different groups. Generally they are temporary larval organs, and it can be easily shown that they are not in all cases homologous organs. In those amphibia which have sucking disks these organs are undoubtedly ectodermal in origin. In the teleost *Echeneis* (*Remora*) the great sucking disk — an adult organ in this case — is developed from the anterior end of the dorsal fin. Ascidians also develop sucking disks; they too are ectodermal.

As to the function of these organs all authorities seem to be agreed. Their function is to attach the young fish to some solid object. As a rule the organ is well developed when the fish is hatched and the little fish attaches itself immediately. This is true at least of *Lepidosteus* and *Amia*. In *Amia* the organ serves to keep the young fish attached for about a week; in *Lepidosteus* about two weeks.

Whether the organ has now or ever has had any other function has not been shown. It has been suggested that they may serve to convey some sort of nutriment to the digestive tract but we have found no proof of this. It has also been suggested that

they are modified sense buds but this is certainly not the case in *Amia*. But the fact that in *Acipenser* the adhesive organs become tactile organs, put with von Kupfer's statement that the adhesive organs and the hypophysis have a common anlage may suggest some remote connection with the nervous system.

As to the phylogeny or meaning of these organs facts are too few to form a basis for any very extensive or certain conclusions. In the various species of ganoids they may have a common function; whether they have a common origin is doubtful. They do not have a common fate. In *Amia* they disappear entirely. In *Lepidosteus* they leave only a useless rudiment. In *Acipenser* they become the barbels. This last fact may furnish a clue to the origin of the barbels in the teleosts as suggested long ago by Balfour. The adhesive organs of ganoids — if we may base our conclusions on their development in *Amia* — are not homologous with the sucking disks of the amphibia or the teleosts. So far as we can see now these organs were developed independently in different groups of animals. But living forms of ganoids are so few and so aberrant that any conclusions based on the study of these forms alone will probably be only tentative.

Summary.

The development of the adhesive organs in *Amia* begins about 70 hours after fertilization when the embryo extends over about 160 degrees of the circumference of the egg.

They appear as paired thickenings of the dorso-lateral portion of the anterior end of the foregut. They are therefore entodermal in origin.

They grow out as paired diverticula of the foregut, break through the epidermis, become greatly subdivided and form a horseshoe-shaped structure at the end of the snout.

Histologically they consist of high columnar epithelial cells. These cells are at first heavily laden with yolk granules especially at their base. They contain a reticulum coarser at the base. Metabolic activity is at first greater at the basal end of the cells but later it is greater at the distal end.

The function of these cells is undoubtedly to secrete a mucous substance by means of which the young fishes attach themselves

to plants or other fixed bodies. They function as secreting organs till they sink below the epidermis.

The organs reach the highest stage of their development just after hatching. They are functional for about a week and disappear in two or three weeks. The mode of disappearance is probably by cytolysis.

LITERATURE.

Agassiz, Alexander.

'78 The Development of Lepidosteus. Proc. Am. Acad. Arts and Sc. Boston, Vol. 13, p. 65–76.

Balfour, F. M.

'85 Treatise on Comparative Embryology. London, Vol. 2, p. 108, footnote.

Balfour, F. M., and Parker, W. N.

'82 On the structure and development of Lepidosteus. Phil. Tr. London, Vol. 175, pp. 359–442.

Dean, Bashford.

'96 On the larval development of Amia calva. Zool. Jahrb. Abt. f. Syst., Vol. 9, pp. 639–672, Taf. 9–11.

Ehrenbaum, Ernst.

'94 Beiträge zur Naturgeschichte einiger Elbfische (Stör). Wiss. Meeresuntersuch., Bd. 1, Heft. 1, Kiel und Leipzig.

Eycleshymer, A. C., and Wilson, J. M.

'06 The gastrulation and embryo formation in Amia calva. Am. Jour. Anat., Vol. 5, pp. 133–162, 4 pl.

Kupfer, C. von.

'93 Studien zur vergleichenden Entwicklungsgeschichte des Kopfes. Heft 1. Die Entwicklung des Kopfes von Acipenser Sturio. München.

Phelps, Jessie.

'99 The development of the adhesive organs of Amia. Science, N. S., Vol. 9, p. 366.

Salensky, W.

'82 Recherches sur le developpement du Sterlet (Acipenser ruthenus). Archives de Biol., Tome 2.

Ziegler, H. E.

'02 Lehrbuch der vergleichenden Entwickelungsgeschichte der niederen Wirbeltiere, Jena.

ABBREVIATIONS.

a.o.	adhesive organs.	*g.en.*	gut entoderm,
br.c.	brain cavity.	*h.b.*	hind brain.
c.	cœlom.	*ht.*	heart.
ch.	notochord.	*mes.*	mesoderm.
d.ec.	deep ectoderm.	*n.*	nasal pit.
en.	entoderm.	*o.v.*	optic vesicle.
f.b.	fore brain.	*p.*	pigment.
g.	gut.	*p.cb.*	pre-cerebral mass.
g.c.	gut cavity.	*s.ec.*	superficial ectoderm.
g.d.	gut diverticulum.	*y.m.*	yolk mass.

EXPLANATION OF PLATE VI.

Figures 1–6 are magnified about 50 diameters; figures 7–11 about 450 diameters.

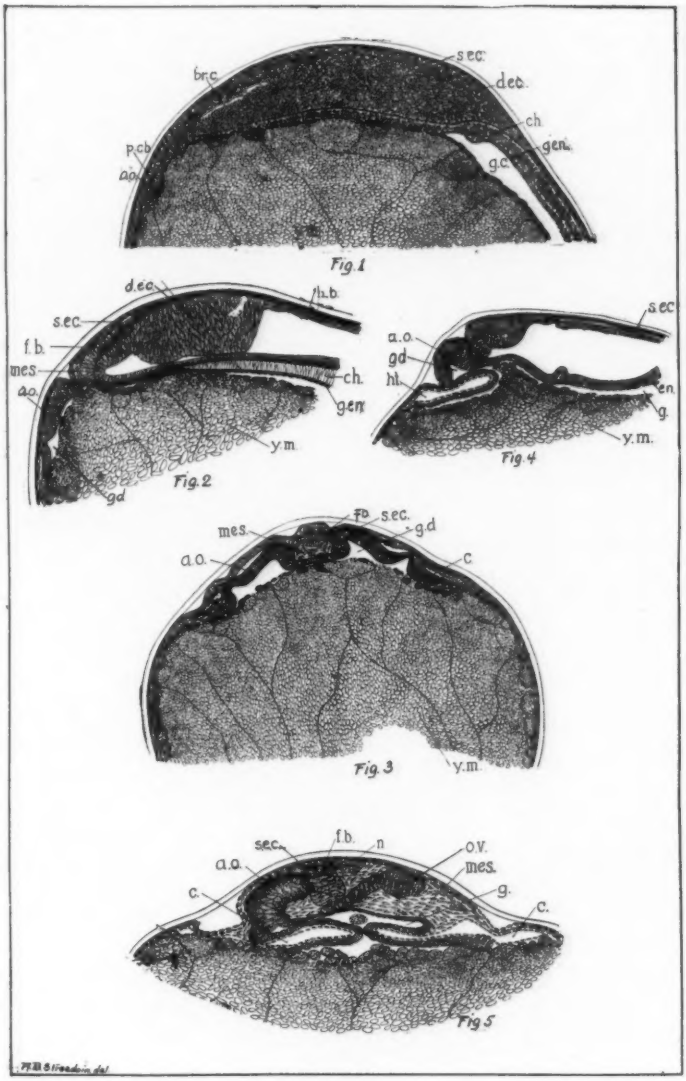
FIG. 1. Median sagittal section of the anterior portion of an embryo about seventy-two hours after fertilization, showing the anlage of the adhesive organs.

FIG. 2. Oblique sagittal section of anterior portion of embryo about ninety-five hours after fertilization.

FIG. 3. Transverse section through an embryo of same age, showing paired diverticula of the foregut which become the adhesive organs.

FIG. 4. Sagittal section of an embryo one hundred and ten hours after fertilization showing median portion of the adhesive organs and the heart.

FIG. 5. Obliquely transverse section of an embryo one hundred and twenty-five hours after fertilization passing through the anterior margin of the adhesive organ on one side and the optic stalk and vesicle on the other.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s.ec.
d.ec.
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a.o.
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Fig. 1
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h.b.
s.ec.
f.b.
mes.
ch.
a.o.
gen.
y.m.
gd.
Fig. 2
s.ec.
a.o.
gd.
ht.
en.
g.
y.m.
Fig. 4
FD.
s.ec.
mes.
g.d.
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Fig. 3
y.m.
sec.
f.b.
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Fig. 5
PHS Freedom del

Explanation of Plate VII.

Fig. 6. Sagittal section through an embryo a few hours older than that shown in Fig. 5, showing one of the adhesive organs cut lengthwise.

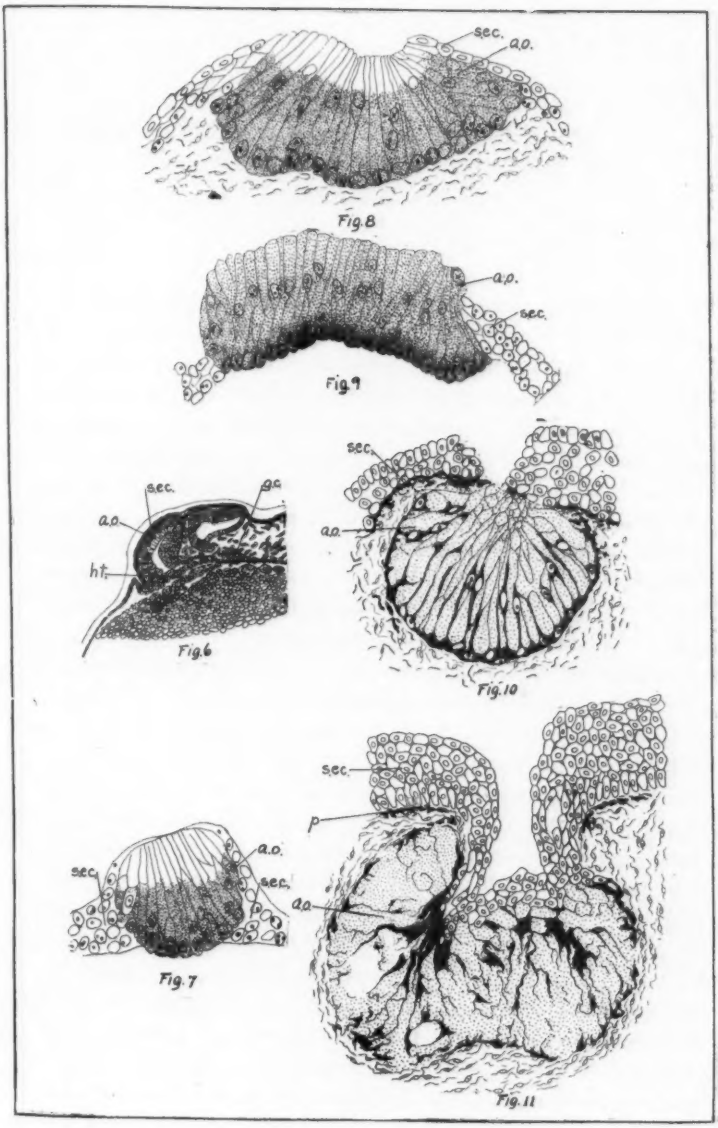
Fig. 7. Section through a single disk of the adhesive organ of a larva in which both head and tail are free from yolk.

Fig. 8. Section through a single disk of a larva about 8 mm. in length.

Fig. 9. Section through a single disk of a larva about 9 mm. in length.

Fig. 10. Section through a single disk of a larva about 14 mm. in length.

Fig. 11. Section through a single disk af a larva about 20 mm. in length.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a.o.
Fig. 8
a.o.
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ac
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Fig. 6
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a.o.
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Fig. 7
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p
a.o.
Fig. 11

SOME FURTHER RECORDS CONCERNING THE PHYSIOLOGY OF REGENERATION IN TUBULARIA.

T. H. MORGAN.

The following experiments are in the main continuations of previous ones carried out to test further certain results whose interpretation was in doubt owing to insufficient data. The main problem concerned the influence of regeneration at the oral end on the rate of regeneration at the basal end. Experience shows that caution must be used in interpreting the experiments that bear on this point, because, in the first place, the rate of regeneration is greatly influenced by slight changes in temperature, and therefore accurate results along these lines should be carried out in the future in water kept at a uniform temperature. The difference of temperature of day and night is a serious obstacle when dishes are kept at room temperature. In the second place the aeration of the water is also an important factor and should be controlled. The extent of surface exposure of the water, the number of pieces in a dish, *and the number of other organisms attached to the stems*, also influence the time of regeneration, in the latter cases by utilizing the oxygen or by fouling the water. In the third place there can be little doubt that an internal "condition" of the colonies plays a rôle in the result. Control pieces from the same colony should always be present, but even this precaution is not entirely sufficient, since different stems of the same colony may differ in their internal conditions.

Despite these difficulties some general conclusions may be drawn, although certain of the experiments will be more profitable when the external conditions are more fully controlled. By keeping the dishes surrounded by running water I have met some of these difficulties in many of the experiments where rate of regeneration is involved.

THE SIMULTANEOUS DEVELOPMENT OF ORAL AND BASAL POLYPS.

As previous work has shown, the "rule" for pieces of the stem of *Tubularia* is for the oral primordium of the polyp to develop first, and then, after several days for the basal polyp to develop unless stolons have already appeared; yet not infrequently the oral and basal ends develop simultaneously. In a few instances the basal hydranths may appear before the oral. It was first shown by Driesch and afterwards confirmed by myself that the time of appearance of the oral hydranth depends on its distance from the oral end. The same relation is observed for basal polyps also. It is therefore of some interest to compare the time of regeneration of oral and basal polyps in those cases where both polyps appear simultaneously to see if any acceleration or retardation can be observed.

In one case after 50½ hours 23 of 75 pieces showed the beginning of oral primordia. There were amongst these, three "double" pieces, *i. e.*, pieces with both oral and basal primordia. In two of these the oral end was more developed; in one the basal end was ahead. After another 24 hours there were 43 oral primordia, of which 21 were on "double" pieces. There were also 3 pieces with only basal primordia. Thus at first there was an excess of oral development, but the number of basal primordia increased at the second observation. A large number of pieces had not produced as yet the oral primordia, and three of these pieces had basal primordia only. The results show that both ends may develop simultaneously, and in such cases the rate of development may not be behind that when only one end develops. It is evident therefore that, if the stimulus to develop is present, two polyps may develop at the same rate as does a single one.

In another case, after 45 hours, 56 of 92 pieces had oral primordia only. Twenty-four hours later there were 28 double pieces. In another case after 45 hours, 27 of 55 pieces had oral primordia only. Twenty-four hours later there were 27 oral primordia, and in addition 18 double pieces.

In another case, after 48 hours, 10 of 29 pieces were double; of these 6 were equally developed at each end, 3 had the oral end ahead and 1 the basal. In another case at another time, after

24 hours 10 of 55 pieces showed only oral primordia. Twelve hours later 29 of 55 pieces had oral primordia, and of these 3 pieces were double — two of them having the basal end ahead.

Three other cases are summarized in the table.

48–50 HOURS.

Oral.	Double.	Basal.	Nothing.
10	3		4
19	14	5	
9	27	4	

These facts show that despite the "rule," the number of double and basal polyps is considerable. Pieces similar to these must be present when the oral ends of pieces are tied. Consequently the rate of basal development of many of the tied pieces may have no connection with the tying of the oral end. Nevertheless comparisons with the control show unmistakably that the percentage of basal polyps is greatly increased by oral ligatures.

In order to see whether the development of the basal polyps in double pieces is accelerated when the basal and the oral ends develop simultaneously, pieces of moderate length were cut off from the distal end of stems and the basal continuations of these pieces were also kept and a record made of the time of their oral development.

In one case after 26½ hours there were faint indications of oral primordia on 15 of 34 of the distal pieces. At this time 5 of the basal pieces also showed oral primordia. Seven hours later the former pieces had 21 oral, the latter 6 oral primordia. Fifteen hours later the former had 29 oral, the latter 15 oral primordia or polyps and one double primordium. The same proportions were found 48 hours later. In this case no double pieces developed at first among the distal pieces, although the proximal pieces early showed primordia. This negative result shows that the basal development of double pieces is not necessarily correlated with the oral development of their basal partners.

In another case, of 50 distal pieces, after 26½ hours, 14 had oral primordia, the 25 basal pieces had 10 oral primordia. Seven hours later the former had 25, the latter 18 oral primordia. Twenty-seven hours later still the former had 32 oral primordia and one basal primordium, the latter 23 oral and one double. Later the same relations held.

In a third case, after 24 hours, 6 of the distal pieces had oral primordia, and none of the basal pieces had developed. Twenty-four hours later the former had 32 oral primordia and one basal. Some of the basal pieces had developed. Twenty-four hours later still the former had 33 oral, 3 double and one basal primordium ; the latter 8 oral.

In a fourth case, after 48 hours, the distal pieces had 16 oral and two double premordia ; the basal pieces showed 11 oral, 4 double and 6 nothing.

In a fifth case, after 48 hours, the distal pieces had 28 oral and 13 double primordia ; the basal pieces 12 oral and one double primordium.

The results seem to show no acceleration owing to oral development, and on the other hand no necessary retardation if both primordia start at the same time. Local factors must therefore determine the results. Most of the distal pieces served also as controls in cases in which the oral ends of other pieces had been tied. In all such cases there was a very great excess of basal primordia in the orally-tied pieces, showing that there is a distinct effect produced in this way. It would seem therefore that despite the fact that both oral and basal polyps may develop simultaneously, the oral development, if it gets the start, inhibits to some extent the *beginning* of basal development. The nature of this influence is still obscure.

Are the Changes in Isolated Pieces Local or General?

Pieces were tied near the oral end and then after a number of hours as much of the basal end as would contain the primordium of the polyp was cut off. In the first experiment the basal cut was made ten hours after tying. Twenty-three hours after the beginning 3 of 9 pieces showed basal primordia. In the control (tied but not cut) 3 of 9 pieces also showed basal primordia. The removal of the basal piece had not, within a total of twenty-three hours, delayed the basal development. In other words the cut end did in thirteen hours what it took the control twenty-three hours to do. This means that the rate of regeneration was accelerated by changes taking place in the pieces at least some little distance from the cut end. No doubt the development would have

been delayed if a larger basal piece had been cut off, but unfortunately no experiments were made to show this. It is however probable that the changes are especially confined to the basal region near the cut end and are less at more distant points.

The converse experiment consisted in cutting off pieces of the oral end. The basal end was tied in the first experiments, although this is not necessary, because tying the basal end does not accelerate the oral development.

When the oral ends were cut off after ten hours the six pieces showed, 23½ hours after the beginning, 3 faint oral primordia. The 6 controls had all oral primordia. After 72 hours all the 6 cut-off pieces had oral polyps, while only two of the controls had oral polyps and two oral primordia. The cut-off pieces appear to have fully caught up with the controls. The data are indeed very few, but the results confirm earlier ones thus obtained. In order to see whether when both ends of a piece are closed any processes take place that accelerate development if the ends are later exposed, the following experiment was made. Both basal and oral ends were tied and later the piece was cut in two in the middle. In order to ascertain the rate for normal regeneration control pieces were cut off and left open. These developed oral primordia after 19 hours in 9 of 14 pieces. These pieces were then also cut in two in the middle. After 55 hours (from the time of tying) the basal halves of the tied and cut set produced 5 oral primordia, while of the control 6 had oral primordia, 2 basal primordia, and one was a double piece. The results indicate that when a piece is closed at both ends no changes take place in it that accelerate the development in the middle of the piece. The result may throw some light on the preceding experiment, and if so, shows that the changes that there took place were due to the open end.

Is Basal Development Accelerated by Allowing the Oral End to Begin its Development?

In one of my previous papers[1] I gave the results of an experiment in which pieces were tied near the oral end after having been left open for several hours. The experiment was made in

[1] *Journ. Exp. Zoölogy*, II., 1905.

order to see if the amount of materials set free by the breaking down of the ridges near the oral end (preparatory to the development there of a polyp) has an influence on the rate of basal development. As a control some pieces were also tied at once at the same level. One such experiment is shown in the next table.

AFTER 48 HOURS.

Control.	Tied after 6 hours.	Tied after 20 hours.
11 Basal	8 Basal	3 Basal
2 Nothing		

Another experiment gave similar results. In a third experiment pieces, tied near the oral end after 9 hours, produced basal primordia before those tied at once.

In this third experiment there was perhaps some indication that when pieces were left open for a time at both ends and were then tied at the oral end the basal polyps developed sooner than when the oral ends were tied at once. This result I had obtained before. The three following experiments do not give the same result.

Of 12 pieces tied at once at the oral end, 2 had basal primordia after 48 hours. Of 11 pieces tied after 26½ hours 3 had basal primordia. After another 24 hours the former had 5 primordia and 6 polyps, the latter 10 basal primordia (and one nothing). These were less advanced than those tied at once.

In another experiment 16 pieces were tied at once near the oral end and produced after 48 hours 14 basal primordia. Of 8 pieces tied after 26 hours 6 had basal primordia. The evidence seems to show that no retardation occurs (or very little) if the oral ends are tied after 9–24 hours as compared with pieces tied at once, and there may be an actual, *i. e.*, an absolute acceleration.

The results of another experiment are also still doubtful after repetition of it. Pieces were left open for several hours and a ligature was then tied near the oral end in some of the pieces, and in others near the basal end. The time of development of the basal primordia was noted in each case. Previous experiments had given some indication that the longer basal pieces produced primordia before the shorter ones. It seemed not improbable, if this were really true, that the amount of material set free

from the oral end accelerated basal development, for more of it would be shut off in longer pieces, although not proportionally more. The new experiments showed that in some cases the pieces ligated nearer the basal end developed as soon as those ligated more orally. Moreover the ligature itself if too near the basal end may interfere with development there. It appears then that it is still unsafe to draw any conclusions from these experiments.

Stolon Formation.

Stolons develop after several days in a small percentage of cases from the basal ends of pieces lying on their side in the dish. More often the basal ends produce heteromorphic polyps. The absence of stolons from the oral ends was first noted by Loeb. The potentiality to produce stolons must of course be present throughout the piece, since they may form from basal cut ends at any level. I attempted in the following way to produce them at the oral ends by first tying pieces so that basal polyps or primordia appeared, and then by cutting off the piece behind the ligature. The presence of the basal polyp might be imagined to make the conditions favorable for the development of oral stolons. This would certainly be the expectation if the polarity of the whole piece is reversed as Loeb supposed. In no case were oral stolons produced in this way, even when the pieces with basal polyps were cut off near to the polyps.

In one case the pieces were cut off below the ligature 10 hours after tying. After 48 hours from the beginning there were 3 oral primordia and no basal. Other pieces had been cut off after 24 hours (when the ligatured controls showed 10 of 27 pieces with basal primordia). All 14 pieces showed oral primordia, and 8 of these had primordia also at the basal end more developed than those at the oral end. The presence of basal primordia had not delayed the development of oral primordia. In fact the latter appear to have been accelerated — not however necessarily owing to the basal development but to other changes in the piece.

It is probable that external factors combined with the internal factor called polarity has something to do with stolon-formation. But even when the oral ends of pieces with basal polyps were

covered with sand or were thrust into little heaps of sand no oral stolons developed.

Influence of Diluted Sea Water.

Some observations of C. D. Snyder on *Tubularia crocea* of the Pacific Coast seemed to him to indicate that in dilute sea water more basal polyps are produced than in normal sea water. In several experiments that I made with *T. crocea* of Woods Hole in which the sea water was diluted no more basal polyps developed than in the control in sea water. The occurrence of basal polyps is so variable that only a thoroughly controlled set of experiments would suffice to prove that dilution affects basal polyp formation.

Direction of Currents.

In *Tubularia* a current passes up one side of the hollow stem and down the opposite side. When more than one dissepiment is present there may be two currents in one direction and one or two in the reverse direction. Those who maintain (Goebel and Loeb, for example) that currents may determine polarity might hope to find in the direction of these currents in *Tubularia* a solution of the problem of polarity. In fact Loeb has suggested such an hypothesis. Since however there is a current in each direction it is difficult to understand how this idea could be supposed to account for the difference in the behavior of the two ends, unless indeed there is a dorsal and a ventral side to *Tubularia* (which seems to be radially symmetrical) so that the dorsal side of the oral polyp is on the opposite side from that of the basal polyp. This supposition is not however in harmony with the heteromorphosis in other forms, such as the earthworm or planarians. In these the ventral and dorsal surfaces are the same in the old and in the heteromorphic structures. The only alternative would be to expect, if the currents in Tubularia are a factor in the direction of regeneration, to find that the current near the basal, or heteromorphic polyp is reversed in direction as compared with that going to the oral polyp.

I have examined the direction of the current in a number of long double pieces, and have found that the direction is continuous along each half of the piece and not different at the two ends.

This observation sets aside, I think, any attempt to explain polyp-formation on the physiological basis of the direction of the currents. The inadequacy of such an explanation is also readily seen when short pieces with "double" partial hydranths are examined, in which the continuous current is readily demonstrated.

"Polarity."

In an earlier paper I have pointed out that experiments show that there exists a graduation in the materials of the stem of *Tubularia*, and that on this as a basis we may attempt to adjust our conception of polarity. This gradation can be seen structurally in the change in the thickness of the walls and in the histological character of the cells of different levels. Physiologically it is shown in the more rapid regeneration of the cut surfaces the nearer they are to the distal end. This difference in rate I supposed might be due to the greater amount of "hydranth-forming" materials[1] near the distal end, or more correctly to the less differentiation of the more distal parts. It was perhaps unfortunate to have used the words hydranth-forming materials, for it might readily be inferred that I meant to refer to formative materials or substances as such, *i. e.*, independently of the differentiation that decreases in amount distally. A careful perusal of the text, especially of later papers, will show that I had not so much in view the presence or absence of peculiar and unknown "stuffs" (a view I have often disputed) as the direction that differentiation had taken in different regions. The more distal parts of the stem are less specialized as storage and supporting tissues than the basal parts. The distal region, having as it were, less to undo, develops more quickly into a polyp. This sort of difference of materials, with its concomitant physiological differences, may furnish a basis for that particular condition

[1] By hydranth-forming materials I meant not reserve stuffs or "organ-forming substances" in the sense in which some embryologists of the preformation school use these terms, but rather materials that have already been differentiated into a part of the anterior end of the body or head. The phrase is more applicable to cases where the head is not sharply marked off from the trunk as in the earthworm, in planarians, etc. That the direction in which differentiation of a tissue has taken place is an important factor in determining the future course of regeneration of that tissue is familiar. By generalizing this fact I have attempted to make it the basis for the phenomena of polarity.

that we call polarity. These material differences with their correlated physiological differences lead to differences in the behavior of the two ends, for, while the material exposed at any cross-cut is the same at both ends, it behaves differently on account of the relation of the end to neighboring regions. This relation may be thought of as one of direction and this is polarity.

It has seemed to me possible that the relation of the parts to each other might be expressed as the relative condition of tension — in a physiological sense — that exists between the different parts. Further analysis has led me to think that behind this relation there is a more subtle one and that irritability is the physiological factor that regulates the behavior of the cells in development and in regeneration. Even the differentiation of the different regions must be supposed to be due to their relation to neighboring regions. In fact, one of the first and most obvious changes that takes place in cells when removed from contact with their fellows is a loss of differentiation, followed by a re-differentiation in relation to a new terminal condition. Polarity therefore in the last analysis stands for a graded relation resting on a condition of contractility (tension) that exists between different levels.

The Cause of the Delay in Basal Polyp-formation when the Oral Polyp Develops First.

In my last two papers on Tubularia I have laid perhaps undue emphasis on the question as to how the basal development of a piece is accelerated when the oral end is tied and conversely as to how the basal end is retarded when the oral end develops. The problem interested me because of its apparent wider bearings; for it seemed possible in this case to test by suitable experiments whether the result could be explained on purely chemical grounds or whether a different principle was involved. The attempt to find a sufficient chemical stimulus does not appear to me to have been successful, for the confessedly incomplete data on which my conclusion provisionally rested has not withstood a second attack. It seems to me now not improbable — and more can not at present be said — that the retardation of the basal development is directly owing to the formative changes taking place at the oral end.

The simultaneous development of oral and basal polyps that sometimes takes place indicates that there need be no absolute antagonism between the development of polyps at opposite ends, but only that such an influence tends to inhibit the beginning stage of the polyp. It is also significant, I think, that the basal development has a smaller retarding influence on the oral development than vice versa, as the experiments show. This means apparently that the locally stimulating factors have a stronger influence on an oral end, due possibly to the direction of the gradation in the pieces. Other experiments show that this direction is a factor in determining the rate. It may seem that this view could be tested by a comparison of pieces of different lengths, for a greater influence would be anticipated in shorter pieces, but it is difficult to make such a test since the decrease in the rate of response when the cut lies nearer the base seriously interferes with a fair comparison being made.

The Factors Involved in the Closure of the Open, Cut Ends.

The extremely rapid closure of the cut end of a stem by means of a plate of cells that advances diaphragm-like from the cut edge, has always excited my interest, because it throws a good deal of light on the way in which movements of materials may take place without any proliferation of new material entering into the process.

Renewed study has shown that the oral and the basal ends of pieces close at the same rate ; that the edges of oral, cut surfaces from the distal region of the stems close approximately at the same rate as oral surfaces near the basal regions ; that the time of complete closure depends on the size of the piece, smaller pieces closing sooner, since the rate of advance of the edge is about the same in all ; and that the addition of salts and of sugars to the sea water, if not so great in amount as to involve serious changes, affect very little the rate of closure. Surface tension therefore seems inadequate to explain the results, although surface tension may play a minor rôle as a part of the stimulus to contraction.

Sections and surface mounts of different stages in the process of

closure, disclose several points of interest. The membrane, that forms and advances ring-like toward the center, is relatively thick and is composed of a large number of cells. The slight withdrawal of the cœnosarc from the cut end is insufficient to account for the presence of so many cells, and the only interpretation that remains is that the cells of both ecto- and endoderm must be drawn towards the cut surface for some little distance from the ends, although the closure of quite short pieces shows that the result may also be attained by a shorter length being utilized. This involves a greater decrease in thickness of the neighboring cœnosarc wall.

What factors are involved in the closure? We may suppose that the stimulus of the sea water, or surface tension, or the loss of contact relations of the material, with its concomitant change of tension relations, is the initiatory stimulus. The last seems to me the main factor for reasons given in previous papers. The tension equilibrium lost, contraction follows. The cut edge contracts as a whole, and as a result of the intimate fusion of the outer layer of the ectoderm with the perisarc the diaphragm-like membrane develops; for if separation from the perisarc is first accomplished a different kind of closure takes place. As the process continues the cells are drawn out bodily over the transversely closing membrane. Despite the rather intimate union of the cells with each other they not only change shape but shift their relations to each other, and while at first a considerable number of cells reach the edge of the closing membrane, their number becomes fewer as the opening gets smaller.

Our analysis leads to the conclusion that the closure is a contractile process of the living substance.

When closure is completed the relation of the parts to each other is still an unstable one for the end of a piece. This leads to further changes in conformity with the irritable nature of the materials and a new polyp develops in consequence. If our analysis is correct we are led to the view that the essential factor in the closure of the stem is the irritability of the protoplasm which leads it to undergo movements (through its power of contractility) of a peculiar kind. This same process, that we find reduced to its simplest term in the closure of the stem, is the mainspring

also of all or most of the formative changes that follow. Irritability therefore is the essential factor in formative action and leads first to change in position of the cells and ultimately to their differentiation. Yet the process of closing gives every appearance of taking place independently of the cells as units; for the movements *appear* to take place in the material as a whole and not to be the sum total of a vast array of cell-adjustments. In other words the cells adapt themselves as best they can to the mass action that is going on and do not take the initiative in the process. Nevertheless it is unsafe to argue from such gross effects that cells have no independent functions. Their power to divide at different times, and the cellular limits of differentiation, show their independence in certain respects. If, however, as seems probable, the cells are united by a meshwork of protoplasm the irritability of the living material may act without heed to cell boundaries.

The Nature of Formative Action.

After the closure of the end of the stem of *Tubularia* the condition is still one of unstable equilibrium; by which I mean that the stimuli received at the closed end cause further changes in the relation of the end to the rest of the piece. The stimuli may be entirely or largely internal, resulting from an unstable termination. In *Tubularia* both external and internal factors may act. This relation leads to the formation of a new polyp in which the relation of the materials to the outside world and to the rest of the piece is a stable one. If my analysis of the factors involved, first in the closure of the stem, and later in its further elaboration into a polyp, is correct we are led to the view that the essential factor is the irritability of the material that determines the location of the formative changes. As I have given recently (the Seventh International Congress, August, 1906) my reasons for coming to this general conclusion I shall not enter further into the argument here, but desire only to bring the interpretation into connection with the present results.

The modern school of developmental mechanics has sought to explain formative actions as the result of familiar chemical and physical phenomena but has not met with any marked

success in explaining such actions. The vitalistic view goes to the opposite extreme and postulates unknown or unknowable processes. Both seem to have overlooked the possibility of accounting for formative changes by the familiar processes of irritability and contractility, that appear to be fundamental attributes of living materials. These cannot be reduced as yet to any known chemical or physical phenomena, but neither does it follow that they belong to a vitalistic category.

To sum up: the formative principle as seen in development, growth, and regeneration, appears to be an expression of the irritability of the living material. The problem of formative action is therefore intrinsically one of response to external stimuli or to internal relations between the parts through the agency of irritability. Contractility is one of the most usual methods of responding to the condition of stimulation that exists in a given region, while differentiation follows quickly in its train. The formative principle is the outcome of a factor that is not one of the familiar chemical or physical events.[1] This conclusion does not force us into vitalism; for until we know something of the nature of irritability it is premature to insist on referring it to any larger category. As yet we are only at the threshold of a knowledge of formative changes.

[1] To those who refer the varied manifestations of irritability to a psychical principle the problem of development will appear to belong to that category; but to those who see in irritability only a "physiological process," the same problem will seem to be physiological. The distinction may be verbal and conventional.

THE CAUSE OF THE PRODUCTION OF "DOWN" AND OTHER DOWN-LIKE STRUCTURES IN THE PLUMAGES OF BIRDS.

OSCAR RIDDLE.

A recent paper ('07) by Dr. Lynds Jones makes clear the morphological relations of "down" and definitive feathers. Jones' work, some experimental results already reported by the writer ('07, '08), together with some hitherto unpublished data bearing on the physiology of avian plumages, now enable us to make some fairly definite statements concerning the causes which lead to the production of "down." A number of observations and experiments on the relations which exist between the *rate of growth* and the *character of the feather structure produced* — pennaceous or plumulaceous — also suggest some interesting conclusions. It will be shown later that results from these various angles of approach center about a common point.

Jones' studies demonstrate positively that "the first down and its succeeding definitive feather are produced by one continuous growth, and therefore cannot be regarded as two distinct feathers. The first down is the plumulaceous tip of the first definitive feather" (p. 17).

This writer has however failed to homologize the modified region which connects the two parts of such a feather with anything already known; and, apparently he has not perceived the actual cause of this modification. To point out the homologies and state the cause of the various modifications found in the down; to show how the "down" and the plumulaceous proximal parts of pennaceous feathers are related to their rate of growth; and in connection with this latter point to put forward a general theory of the significance and relations of all plumulaceous and pennaceous feather structures, is the purpose of the present paper.

Homologies of the Modified Region of the Down.

Anyone who is familiar with the several forms of feather defects or fault-bars which have elsewhere been fully described by the

writer ('07, '08), will find that Jones' plates and description practically prove the existence of another variety of this same defect; in other words one may say that the modified region of the down — the "quill" — is a variety of defect which we have already recognized in other situations, but which here occurs in a very uniform way — at a point near the end of the feathers of the first plumage. The fact is, simply, that the defective region falls so close to the end of the feather that it does not include any of the shaft of the feather to which it belongs.

There can be, I think, no question as to this interpretation. The exact conditions met with in the defects or fault-bars already referred to are to be found in the modified region of the down: (1) In the modification or absence of barbules (Davies '89, Jones); (2) in the occasional fusion of the barbs (Klee '86, Davies, Jones); (3) in the diminished differentiation (Davies, Jones) and growth (Davies); (4) in a defective development between a more distal and a more proximal part of the same feather (Jones).

Now if the "quill region" of the down is the morphological equivalent of the fault-bars (which are often produced "normally" and which have been produced at will experimentally, at all levels in the definitive feather), we should expect to find that the two have the same or a similar cause. What is the evidence for such a common cause? What causes the production of "down"?

The Cause of the Modifications in the Down.

It has been established beyond question that the several types of fault-bars are produced by insufficient nutrition; even the variations produced in the available food-supply by the daily fluctuations in blood-pressure was shown to be able to leave its mark on feather structure (Riddle '07, '08). What now is the evidence that reduced or insufficient nutrition is the cause of the modification which occurs near the distal ends of the first feathers of birds.

The most highly modified region of the down, *i. e.*, the "quill" region, is produced in all cases so far as I am able to ascertain, after and soon after the hatching of the bird. There are the following reasons for a defective nutrition at that time:

1. The whole source of food-supply for the young bird is now changed. Heretofore, as an embryo it has formed its tissues from substances once assimilated by the mother-bird and stored by her within the egg, from which it extracts them by means of the *yolk-sac*, — a provisional appendage of the gut; henceforth it is subject to the vicissitudes of a much greater and very competitive world for its food-supply; and its *entire alimentary tract*, with its various appendages, new and untried as it all is, must now begin to work — and work properly and successfully — on the hodge-podge of digestibles and indigestibles which here begin their intermittent flow into it. If there is ever such a thing as a "critical period" in a bird's life, indeed it is here! We should have the strangest of miracles performed before our very eyes if this transformation and adjustment were to occur instantaneously and without interruption of any of the nutritive processes of the animal.

2. The skin of the bird is exposed to the chilling and evaporating effects of the air; this doubtless lessens the blood-supply to the integumentary structures. This chilling, moreover, is now of all times the most effective, for, the bird now has the least plumage to help it retain its heat; then, too, the heat radiating surface — the skin — is greater in proportion to the mass of the animal than it will ever be again, and therefore the heat loss at that time is greatest. It is, of course, true that such an organism tends to make good the greater heat loss by increased heat production; but this latter process means at the same time, a greater use and destruction of food at a time when, as stated above, the nutritive mechanism of the animal has not got into full swing.

We are, however, in possession of some direct evidence that a faulty nutrition is the cause of the production of the "down."

1. If a chick is kept continuously underfed from the time of hatching and while in its downy plumage, it will be found that almost all of the feathers (except primaries, secondaries, and a few others) can be kept in the "downy" condition and the bird can thus be made to wear its downy plumage for months (many kept four to five months).

The "quill" region is a part of the feather which "normally" *almost* refuses to grow; by reducing the food-supply during and

after its formation further growth may be absolutely inhibited or stopped. If, therefore, faulty nutrition can and does completely halt the growth at the proximal part of the quill, there is every reason to believe that the same cause *may* have acted as the check upon the growth at the distal and all intermediate parts of the quill; and since it has been shown that the structures pro-

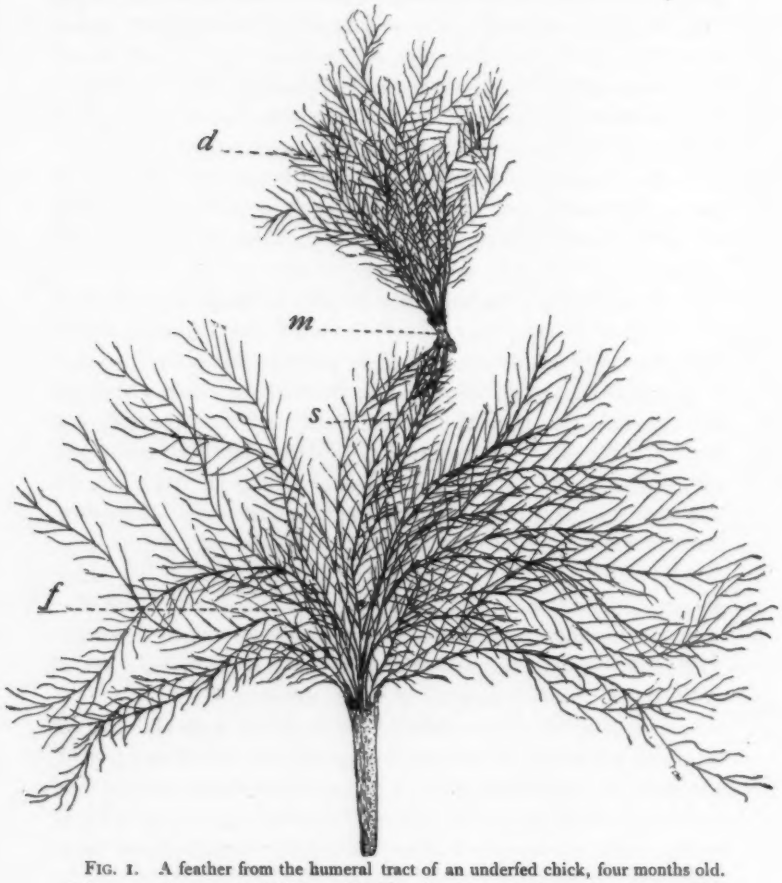

FIG. 1. A feather from the humeral tract of an underfed chick, four months old. *d*, downy portion; *m*, the highly modified basal region of the down, *i. e.*, the "quill." *s*, the barbule bearing shaft of the modified pennaceous feather *f* which grew under "starving" conditions and thus became downy or plumulaceous in character. Drawn with a camera lucida. Actual length of feather 3 cm.

duced in the "down-quill" are in every way similar to those of a series of defects known to be produced by malnutrition, it becomes extremely probable that an insufficient food-supply is he cause of the "quill" formation also.

2. The case just cited is supplemented and strengthened by the peculiar structure of certain feathers from the humeral region (of one of the chicks mentioned in the experiment above) which were able to continue their growth under the conditions of my experiment. These feathers have all the appearance and texture of down, excepting the presence of a very slender shaft. The barbs, however, are not closely set into this shaft as they are in a normally grown feather, but unite with it only at wide intervals. This is clearly an approximation to the conditions in the down where the barbs do not unite at all. The shaft, moreover, bears barbules and these again are exactly like those borne on barbs. Such a feather is shown in Fig. 1.

3. In the plumulaceous basal parts of the feathers of the chick I have produced structural conditions which are in many ways like those of down, and am in a position to state definitely that they were produced by "starving" the bird. In Fig. 2 is shown a section of a feather bearing such downy formations.

4. A fourth line of evidence that the down — or rather its basal portion — is produced under poor nutritive conditions is afforded by the fact that the most emphasized of the down malformations — the horny cylinder or quill — is to be found most frequently among the altricial birds as was pointed out by Jones. Jones does not state that the "quill" is the most extreme modification of these downy structures, but both his work and mine confirm that view.

I quote the following single paragraph which Jones writes on quill-formation, and which seems to include something else quite as important:

"The progress of transition which results in a so-called 'quill' or tube differs in some important particulars from that just given. In the early stages of development no difference is recognizable, but at a little later stage the whole mass of intermediate cells (Fig. 45, Pl. IV., *cl.in*) as well as the sheath cells (*cl.tu*) become much flattened, their nuclei elongated, and their

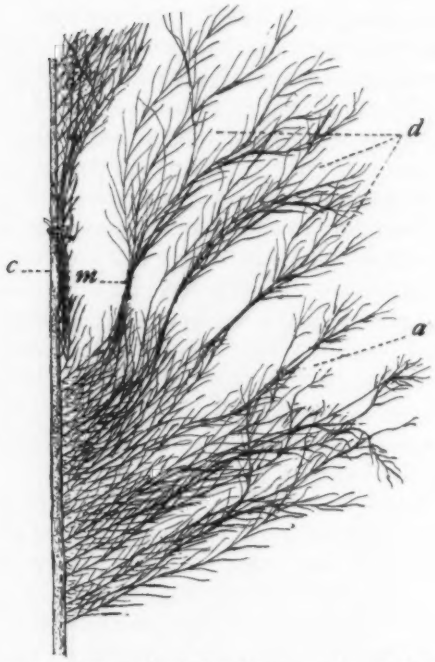

FIG. 2. A section of the proximal plumulaceous portion of a body covert from a chick, showing a modified region with "downy" formations *d*, the much-modified bases of which were produced during a "starving" period of two days. *m*, the horny covering (quill) of a bundle of six barbs; *a*, the abnormal area (fault-bar) produced at another point; *c*, the pigmentless part of the shaft which was grown during this period. Drawing made with camera lucida. Actual length of section shown 2 cm.

cell boundaries lost in a mass of fibrous tissue. Only the row of cells next to the pulp, representing the cylinder cell layer, retains its characteristic shape. At a still later stage in development, represented by Fig. 46, in which the epitrichial sheath is not shown, cornification of the outer rows of cells, representing the region of the sheath cells, has taken place, only suggestions of its original fibrous structure remaining. The outermost intermediate cells have become almost wholly fibrous, narrow spaces representing the position of the nuclei. The process of cornification now proceeds rapidly until practically all of the intermediate cells become cornified, and the cylinder cell layer becomes

fibrous. Fig. 33, Pl. III., represents the final stage in development. That the formation of this horny tube is wholly different from the process by which the shaft and quill of the definitive feather are formed, as described by Davies (p. 594 et seq.), is evident. Instead of being a process designed for the accomplishment of a definite work — the building of shaft and quill — it appears to be due to a lack of differentiation of the cell mass and a short cut to cornification of the tissues induced by a reduced blood supply to this part of the feather during the period when the cells would be showing differentiation if supplied with sufficient nourishment. It is significant that this condition of a cornified ring instead of the normal barb-vanes is more often found among the strictly altricial birds which are hatched in a helpless condition. It is well known that the first few days after the hatching of altricial birds are the most critical days of their lives. During this critical period there appears to be no growth of the down. An American robin which hatched on the fourteenth day of incubation possessed the usual down upon the head and back. These downs made no further growth. It was not until the fourth day after hatching that the skin gave evidence of the beginning of the definitive feathers. On the eighth day after hatching the skin surface was exposed to the drying influences of the air before renewed activity in the feather germ began. During this interval of four days the so-called 'quill' was formed at the proximal end of the down by the rapid drying of the imperfectly formed barb-vane ridges" (p. 13).

I wish to make a number of statements concerning the paragraph just quoted; for, from my point of view, a number of things are here touched — but hardly grasped — and, at any rate, not made really clear.

In the first place the statement is made (here and elsewhere) that in the formation of the "quill" a lack of *differentiation* is the process at fault. My own studies on similar structures — and I think Jones' plates, as well as some points of his description show the same — indicate that while lack of differentiation is undoubtedly a part of the process, a more important part is lack of *growth*. The barbules, for example, do not differentiate, but the cells which should form them do not *grow*. That is to

say, many of the necessary cells never arise at all and those which are to be found in the barbule region never attain the normal size of such cells. Davies recognized, to a certain extent at least, a deficiency of growth of particular parts of this region. He states, "Gewöhnlich verschwinden (in the quill) die Leisten nicht vollkommen, obgleich sie eine bedeutende verminderung ihre Grösse erfahren" (p. 581). On the other hand, one kind of differentiation, *i. e.*, cornification or development of keratin proceeds without interruption in all of these cases.

Jones further states that apparently this "lack of differentiation . . . is induced by a reduced blood supply to this part of the feather, etc." He is here speaking of the conditions in the "quill"-formations only and does not apply this statement to the other form of down modification which he considers (p. 11) the typical one. He does not furnish any evidence for the statement just quoted, and does not refer to the direct and conclusive evidence which my paper ('07) supplies — and I may add that this is still the only direct evidence we have — that a reduced blood supply tends to produce just such feather modifications as are represented in the quill of the down.

Jones' observation that there appears to be no growth of the down in altricial birds during "this critical period" is important and suggestive as supporting the view that wherever we find the "down" we can assert that it signifies defective nutritive conditions in the bird at the time that part of the feather was grown. It is, however, doubtful whether Jones had anything similar to this in mind, for it will be seen that his last word on this subject is that "in the robin (the only specific case cited) the so-called 'quill' was formed at the proximal end of the down by the *rapid drying* of the imperfectly formed barb-vane ridges."

Finally, it should be noted that Jones states that in the case of the young robins the "quill" was formed *during* the fourth to the eighth days after hatching. It seems to me extremely probable that the first four days after hatching were even more important in producing the modification than the four succeeding ones.

Rate of Growth in Relation to the Kind of Feather Structure Produced.

It is well known that "down"—*i. e.*, the distal plumulaceous tip of the feather—and the plumulaceous proximal parts of pennaceous feathers are similar as regards their appearance and texture. They all possess long, slender barbules—usually without hooked barbicles. So far as my observation goes all have the barbules set rather widely apart, and have a fluffy appearance. Since Jones has shown that the "down is the plumulaceous tip of the first definitive feather"; and since in all typical pennaceous feathers we have also a plumulaceous proximal end of the feather, what does it mean in the development of the first feather that two plumulaceous regions are produced with a pennaceous region between?

The writer is convinced that the type of feather structure produced is somehow quite definitely correlated with the relative *rate of growth* at which the various parts of the feather are developed. The following facts and observations are submitted in favor of this view:

1. That the "down" is of slow growth is proved by the works of several writers—Studer '73, Klee '86, Davies and Jones among others—who have shown that in various birds the down begins to develop from the fifth to the eighth day (in the egg) and continues usually fifteen to twenty days, or longer. This, when compared with the growth which succeeds it is obviously very slow. Dwight ('00) also notes that "during the early days of the newly-hatched chick (passerine birds) feather growth is comparatively slow, but shortly it proceeds with marvelous rapidity" (p. 99).

2. If a feather from a juvenal plumage is taken for consideration, it may be said that that part of the feather which lies between the "down" (plumulaceous) and the basal plumulaceous portion of the feather, is grown (rectrices or remiges of ring-dove) at an average of more than twice the rate of either of these extremities.

The slow rate of growth of "down" is self-evident. The following measurements in mm. of a rectrix of the ring-dove are given to show that the above statement is true as applied to the proximal plumulaceous growth.

TABLE I.

SHOWING RATE OF GROWTH OF RECTRIX OF RING-DOVE (*Turtur risorius*).

Days after appearance beyond skin.	1–4	5–8	9–11	12–15	16–20	21–25	26	27	28–32
Length of feather.	22	44	59	78	96	112	115	117	123
Average daily growth.	$5\frac{1}{2}$	$5\frac{1}{2}$	5	$4\frac{4}{5}$	$4\frac{1}{2}$ *	$3\frac{3}{5}$	3	2	$1\frac{1}{5}$

At the point indicated with the star (*), *i. e.*, at about 95 mm. from the distal tip of this feather, it is found that the plumulaceous formation begins. At first only those barbs which lie in the germ opposite to the shaft are affected; but as growth proceeds — and as the *rate of growth diminishes* — more and more barbs become affected.

After having watched the rate of growth of many feathers in chicks and doves only to find that the plumulaceous part always begins at the point of, or after, a considerable falling off in this rate of growth, one is tempted to the conclusion that in these feathers *the two kinds of feather growth, plumulaceous and pennaceous, are merely expressions of slow and rapid growth respectively.*

It is, I think, moreover, quite certain that for many birds the general rule can be laid down that those feathers which as a whole grow slowest have the greatest proportion of plumulaceous growth.

One is led by such considerations to inquire whether all strictly plumulaceous feathers are of slow growth. I know but little of these conditions from personal observation, but the known facts, in so far as I have been able to ascertain them, are in harmony with this view.

It is stated that the ostrich plumes grow at the rate of one inch per week. For such feathers of such birds this is indeed a slow rate — only about 3.5 mm. per day; whereas a little, newly hatched ring-dove will grow remiges and rectrices at from 5 to 7 mm. per day. I feel confident that it will be found that all plumulaceous feathers are grown at a relatively slow rate.

The aftershaft (hyporhachis) which is found in many feathers is another plumulaceous formation and like the others is of slow growth. It seems to me highly probable that a closer study of

the nutritive conditions in this region of feather-germs would reveal the reason for the presence of this feather-accessory in some plumes and its absence in others.

The true quill (calamus) also shares this slow growth of the proximal end of the feather. Indeed it is in the quill that we find the slowest rate of growth to be met with in the whole length of the feather. I am inclined to "explain" the quill as the type of feather formation which results from nutritive conditions which become slowly and progressively poorer; this in turn is able to almost completely stop growth and cell-division, but affects the process of cornification, *i. e.*, keratin formation, to a much smaller extent. It seems to me, too, that our knowledge of feather-growth (quill-formation) in the Japanese fowls, particularly the results of Cunningham's ('03) experiments, and many other facts support this conclusion. Neither the position nor the presence of a quill is "predetermined" in the feather, but both of these are merely marks left along the course of the ebbing tide of a greatly diminished feather nutrition.

The fact that plumulaceous structures do not show the maximum of growth and differentiation (*e. g.*, weaker barbs, and barbules without hooklets) together with the observation that such regions occasionally result from under-feeding (Fig. 1), would seem to lend weight to the view that such regions or such entire feathers are grown under nutritive conditions considerably below the optimum.

It should be remarked that if the view here put forward is correct it would lead us to expect a pretty general occurrence of growth-marks on all feathers which are growing at the time of hatching and soon thereafter. Such marks seem not to have been reported for the rather extraordinary first feathers of the Anserine birds. My own observations on this plumage of these birds are too meager to mention, but it seems quite probable that such marks are much less in evidence there — if they exist at all — than in most other birds. It is conceivable, however, that the young of these birds have a greater quantity of egg-yolk left for their first day or two after hatching; or that they have a considerable store of available fat in their bodies; or yet another means of tiding them over the "critical period" which in these

birds is of course very short. On this point, however, I can furnish no observations of value, and can only say that the exact conditions in these forms are not clear.

It must be left to the further investigations of those who in the course of their studies are able to examine the plumages (particularly the plumulaceous ones) of all of the families of birds to decide whether the theory here advanced of the slow rate of growth of the several plumulaceous formations can be universally and absolutely applied. It is the hope of the writer that some one may have the material and the inclination to put this part of the work here reported to a more rigorous test than the writer's limited material has permitted him to do.

Methods and Materials.

For the experimental part of the work here reported only a few forms have been used; but these few animals have been very closely watched and studied. Nearly all the starving experiments were made on the young of the chick (*Gallus domesticus*), and both on the young and old of the ring-doves (*Turtur risorius*). For the modifications of the nature of the feather growth the chicks are by far the better material. Control experiments were maintained throughout.

It should be said that the under-feeding or "starving" of these animals was usually either accompanied or accomplished by the feeding of the fat-stain Sudan III., which appears to "tie up" the fats of the body. The stain was fed for a purpose not immediately connected with the results reported here, but there is little doubt that it has no specific action on the feathers except in so far as it helps to bring about "starving" conditions in the animal.

Summary.

1. The highly modified region of the "down" is in all respects similar to other feather defects or fault-bars which have already been described as occurring at any and all levels in definitive feathers.

2. Juvenal feathers can by under-feeding be made to persist (chick) in the "downy" conditions practically without growth for several months if the reduced feeding be begun immediately after hatching.

3. Occasionally feathers may be found which have been able to continue their growth proximal to the downy portion despite the inhibitory influences of the lack of food; such feathers have been found to represent a type of structure intermediate to the downy and pennaceous formations.

4. The cause of the modification at the base of the down is to be traced to an interruption, or at any rate to the inadequacy of the nutritive processes of the bird. This interruption is doubtless partly accounted for by the change of source of food from the embryonic to the adult life.

5. The apparent absence of such growth-marks in the feathers of the duck and other anserine birds remains unexplained.

6. The rate of growth of the two ends of a juvenal, pennaceous feather, and of the proximal ends of all pennaceous feathers which bear a plumulaceous proximal portion, is much slower than the rate of growth in the central pennaceous part of the feather.

7. It seems probable that all plumulaceous structures are produced at a relatively slow rate of growth; and also probable that during their growth they have not enjoyed optimum nutritive conditions.

8. The formation of the quill is probably the direct result of a progressive diminution of an already lessened food-supply.

LITERATURE.

Studer, T.

'73 Die Entwicklung der Feder, Inaug.-Dissert. (Philos.) Facult. Bern., 29, p. 2, 1873.

Klee, R.

'86 Bau und Entwicklung der Feder, Hallersche Zeitschr. f. Naturwiss., Bd. 59, Heft 2, pp. 110–156, Taf. 18–20, 1886.

Davies, H. R.

'89 Die Entwicklung der Feder und ihre Beziehungen zu andern Integumentgebilden, Morph. Jahrb., Bd. 15, pp. 560–645, Taf. 23–26, 1889.

Dwight, Jonathan, Jr.

'00 The Sequence of Plumages and Moults of the Passerine Birds of New York. Ann. N. Y. Acad. Sci., Vol. XIII., No. 1, pp. 73–360, 1900.

Cunningham, J. T.

'03 Observations and Experiments on Japanese Long-tailed Fowls, Proc. Zoöl. Soc. of London, 1903.

Riddle, O.

'07 A Study of Fundamental Bars in Feathers, Biol. Bull., Vol. XII., No. 3, Feb., 1907.

'08 The Genesis of Fault-bars in Feathers and the Cause of Alternation of Light and Dark Fundamental Bars. (In Press, Biol. Bull., '08.)

Jones, Lynds.

'07 The Development of Nestling Feathers, Lab. Bull. No. 13, Oberlin College, 1907 (Nov.?).

THE HULL LABORATORIES OF EXPERIMENTAL
THERAPEUTICS AND OF ZOÖLOGY,
THE UNIVERSITY OF CHICAGO,
December, 1907.

THE LIFE HISTORY OF THE CARPENTER ANT.

JOHN LOSSEN PRICER.

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INTRODUCTION.

The studies reported in this paper were undertaken originally with the purpose of applying to another species of ants some of the tests and experiments which have yielded interesting results to other investigators, and incidentally I have been led to endeavor to work out the life history of a colony. In respect to the ecology of my species I have not aimed at completeness in any division, but have taken up whatever was at once most available under my conditions and most promising of results within the time at my disposal.

The work was done in the graduate school of the University of Illinois, as a part of the requirement for the degree of Master of Arts, and under the immediate supervision of Dr. S. A. Forbes, to whom I am deeply indebted for his many kindly and helpful suggestions and criticisms.

MATERIAL AND METHODS.

Two varieties of *Camponotus herculaneus* — *C. pennsylvanicus*, and *C. ferrugineus* — were made the basis of this work. So far as I have been able to learn, these varieties have exactly the same habits, the only difference noted being that *C. pennsylvanicus* is slightly more abundant in this region than the other variety.

The colonies used in my experiments were, for the most part,

collected from various small tracts of woodland within three or four miles of Urbana. These colonies were placed, after collection, in artificial nests of the Fielde type (BIOLOGICAL BULLETIN, Vol. II., No. 2), and were kept throughout the winter in a room of the insectory of the state entomologist, which is on the university campus. This room is heated by steam and was kept during the winter at a fairly constant temperature, ranging from 70° to 90° F. The ants were fed on sweetened water, pieces of insects, cooked lean meat, boiled eggs, etc., and all seemed to thrive perfectly on this fare.

My experiments were modeled after those of Fielde, Lubbock and others, but were usually modified in some details, in order to adapt them better to the species used; and a few experiments were specially devised to follow up and verify the conclusions arrived at. One outdoor colony was studied rather closely from about July 1, 1906, until their activities ceased with the approach of cold weather. All the work was done during the academic year of 1906–07, and all dates given in this paper are of this year.

LIFE HISTORY OF A COLONY.

Believing that a knowledge of the complete life history of such perennial colonies as are formed by the ants under consideration might throw considerable light on other important topics, I have undertaken to do what I could to work it out. The problem is a difficult one to handle in the short time of one collegiate year, and my results are necessarily incomplete.

My methods have been as follows:

1. I captured queens which had not settled in permanent quarters after their marriage flight, and placed them in artificial nests to rear their first season's broad of callows. I compared these small colonies as to number, size and general character of the individuals with many other similar colonies found in natural outdoor nests.

2. Throughout the winter I collected as many larger colonies as possible and carefully counted both the adult ants of all forms, and the larvæ. These colonies were collected when the temperature was low enough to make them inactive, so that by care-

fully chopping to pieces the logs, stumps and trees in which they were found, I was able to obtain the colonies almost entire. Some colonies were counted as they were first picked up, and others were killed in cyanide bottles and counted later.

As a result of the first method I have the following data:

On July 10, 1906, I found a dealated queen of *C. pennsylvanicus* crawling on the sidewalk in the university campus. I placed her in a Fielde nest in a basement room of the insectory and observed her daily throughout the summer and autumn. For the first three days she remained in the light room as if not content with her lot, but she then went to the dark room and on July 18, I saw the first eggs. Eggs were laid, in all, as follows:

July 18	2	July 24	2
" 19	3	" 25	1
" 20	2	" 26	2
" 21	2	" 27	1
" 22	2	" 28	2
" 23	2	August 1	1

These eggs hatched as follows:

August 11	2	August 16	2
" 12	3	" 17	1
" 13	2	" 18	2
" 14	2	" 20	1
" 15	3	" 22	2

Two eggs did not hatch, but either dried up or were eaten. The first larvæ to appear grew very rapidly, almost doubling in size in a single day, and the rate of growth decreased gradually as other larvæ appeared to demand food and care. By September 1, the change in size was scarcely perceptible in a week's time.

These larvæ pupated as follows:

September 1	2	September 10	1
" 3	1	" 12	1
" 4	1	" 16	1
" 5	1	" 25	1

As twelve callows appeared, three more larvæ must have pupated, but the dates of their pupation are not known. The twelve pupæ gave the imago as follows:

September 22.....2	October 2.....1	October 15.....1
" 25.....1	" 5.....2	" 19.....1
" 27.....1	" 8.....2	" 20.....1

A few more eggs were laid during September, so that there were 14 small larvæ in the nest on October 15. These did not grow perceptibly until January, and then only slightly. During that month and at intervals afterwards, the queen laid a few eggs, and by May 1, the colony consisted of 15 callows, 21 larvæ and 8 eggs.

Taking the time required for the development of the first two callows as an approximate average, we have the following periods for the different stages: egg 24 days, larva 21 days, and pupa 21 days, making a total of 66 days from egg to adult. These periods are doubtless all liable to be affected by temperature and other varying conditions, for in outdoor nests some larvæ spend the winter in a state of arrested development, and I have kept one colony — No. 2, Table I. — in an artificial nest all winter in the insectory and no growth could be noticed in the larvæ until about March 1, when they suddenly began to grow at about the usual summer rate. On February 20 I gave five freshly laid eggs to a small colony with neither queen nor larvæ. Three of these hatched March 24, 28 and 30, respectively. The other two failed to hatch. These results show considerable variation in the length of the pupal period.

Another dealated queen of *C. pennsylvanicus* found on the sidewalk July 15 and placed in a nest, began laying eggs five days later, and continued at about the same rate as the one above mentioned, until twelve in all were laid. The first two of these eggs hatched August 13, an incubation period exactly the same as that of the first two eggs of the other queen. At this time the nest was allowed to become too dry and these two larvæ died and four of the eggs were destroyed. Four callows finally reached maturity, however, and the queen laid eggs at intervals throughout the winter, but was not very successful in bringing them to the adult form.

A little sweetened water was kept constantly in the nests of these two colonies and pieces of insects and some other forms of proteid food were occasionally given them, but from the time of

capture until several days after the first callows emerged I did not see either of them take food, neither was there at any time any apparent diminution of their food supply. For several days at a time I gave them only the merest drop of sweetened water, to see whether they would make a meal of it or not, but I could

FIG. 1[1]. A piece of linden bark showing a cavity in which a queen of *C. pennsylvanicus* and her first season's brood of callows were found.

see no evidence that any of it was eaten. This observation, taken together with the fact that a number of outdoor colonies of sizes similar to these were found sealed up in small cavities with no communication with the outside world, as shown in Figs 1 and 2, confirms the conclusions of McCook and others that young queens take no food while rearing their first callows.

In addition to these two colonies, reared from the start in artificial nests, I have collected and counted those represented by Tables I. and II.

[1] The photographs for the illustrations were taken by Dr. C. F. Hottes, Professor of botany in the University of Illinois.

It is evident from the small range in the numbers of workers and larvæ, and from the similarity of these outdoor colonies in this respect to the two described above, that all the colonies rep-

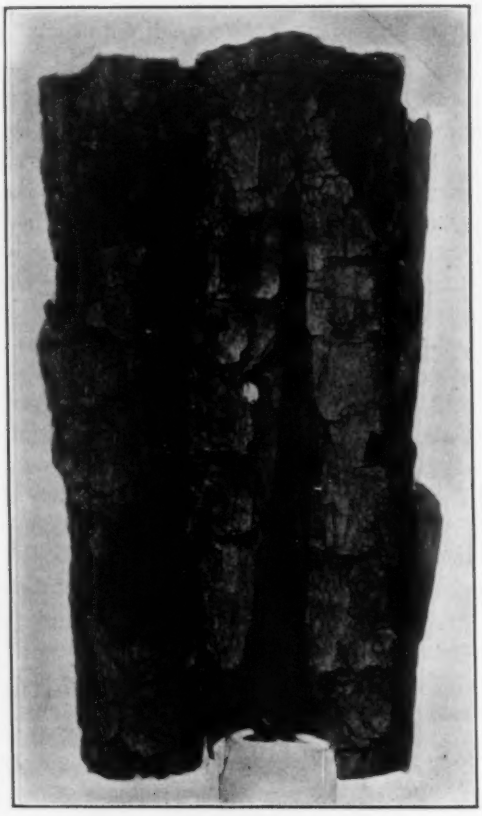


FIG. 2. Same as Fig. 1, showing hole through which the queen found entrance to the cavity.

resented in the tables below were established during the summer of 1906. The difference in the number of individuals in the different colonies is probably due to the fact that the queens left their parental nests at different times, with different amounts of reserve food, and met with various vicissitudes in their efforts to

establish new colonies. Hence we may safely regard these as colonies of one season's development, or one year old colonies.

TABLE I.

SMALL COLONIES OF *C. pennsylvanicus*.

No.	Date.	Situation.	No. Queens.	No. Workers.	No. Larvæ.
1	Oct. 20	Decayed oak stump.	1	27	21
2	Nov. 3	Oak stump.	2	23	32
3	"	Cherry stump.	1	13	18
4	Dec. 28	Linden log.	1	3	12
5	Feb. 9	Ash stump.	1	15	18
6	"	Oak stump.	1	7	10
7	"	Cherry stump.	1	3	12
8	Feb. 16	Decayed oak log.	1	15	18
9	"	Oak stump.	1	22	30
10	"	Linden log.	1	8	18
11	"	" "	1	3	10
12	"	" "	1	4	19
13	"	" "	1	5	11
14	"	Oak stump.	1	11	20
15	"	Ash log.	1	6	9
16	"	Linden log.	1	2	15
17	"	Hickory stump.	1	4	18
18	"	Linden log.	1	7	25
19	Mar. 9	Hickory log.	1	18	32
20	"	" "	1	4	14
21	"	Hickory stump.	1	13	21
22	"	Oak stump.	1	16	17
23	"	Cherry stump.	1	3	8
24	"	Poplar log.	1	4	18
25	Mar. 23	Oak stump.	1	13	18
26	Apr. 6	Linden log.	1	4	12
27	"	" "	1	7	13
28	"	" "	1	21	20
29	"	" "	1	17	20
30	"	Oak stump.	1	9	10
31	"	Linden stump.	1	5	11
32	"	" "	1	9	8
33	Apr. 13	Linden log.	1	9	21
34	"	" "	1	7	13
35	"	" "	1	8	17
36	"	" "	1	3	14
37	"	" "	1	7	15
39	"	Hickory log.	1	19	22
40	Apr. 20	Linden log.	1	2	15
41	"	" "	1	24	30

For the remaining years of the life of a colony we shall have to depend on my second method, and this will give unsatisfactory results, especially because of the limited number of data which I have been able to collect. These data are presented in Tables III. and IV.

In addition to these data I have the following miscellaneous notes :

1. May 20, 1906, I caught a winged queen of *C. Pennsylvanicus*, crossing the sidewalk on the University campus.

2. June 12, 1906, I came upon a hollow tree with a small opening at the base. Around this opening were fifty or more male ants of *C. ferrugineus* in a state of great excitement, and the workers were dragging them back into the nest.

TABLE II.

SMALL COLONIES OF *C. ferrugineus*.

No.	Date.	Situation.	No. Queens.	No. Workers.	No. Larvæ.
1	Feb. 9	Oak stump.	1	15	25
2	"	" "	1	7	19
3	Feb. 16	" "	1	6	17
4	"	Hickory log.	1	9	15
5	"	Linden log.	1	10	21
6	"	" "	1	3	14
7	Mar. 9	Hickory log.	1	7	15
8	"	Oak stump.	1	12	26
9	Mar. 23	Hickory stump.	1	5	13
10	Apr. 6	Linden log.	1	7	12
11	"	" "	1	15	23
12	"	" "	1	4	10
13	"	" "	1	9	19
14	"	Linden stump.	1	11	21
15	Apr. 13	Linden log.	1	6	13
16	"	" "	1	5	11
17	Apr. 20	Cherry log.	1	13	26
18	"	Linden log.	1	2	16
19	"	" "	1	19	30

3. A friend told me of a colony of *C. pennsylvanicus* which inhabited a sill of his house and threw large quantities of particles of wood into his cellar. A large number of winged forms were seen about the outer opening of the nest about the first of July, and again about the middle of the same month.

4. July 6, 1906, I chopped in pieces a small decayed ash log and found a large colony of *C. pennsylvanicus* in it. Besides the workers there were probably 150 males, a large number of larvæ of all sizes, and approximately 200 pupæ, mostly of queens. About 50 workers, 30 pupæ, 20 larvæ and 15 males were taken and placed in a nest in the insectory. All the larvæ died before pupation, but seven queens and four workers emerged from the pupæ and lived through the winter in the nest. The queens emerged on the following dates:

July 25.....1	July 28 1	August 5.....1
" 27.....1	" 30.....2	" 7.....1

TABLE III.

LARGE COLONIES OF *C. pennsylvanicus.*

No.	Date.	Situation.	No. Workers.	No. Wingless Queens.	No. Winged Queens.	No. Males.	No. Larvæ.	No. *X. cava.*
1	Sep. 4	Apple tree.	2,500[1]	None seen	200	150	300	0
2	Apr. 13	Oak log.	3,018	1	196	174	842	27
3	Mar. 9	Oak tree.	2,609	1	207	116	486	4
4	Mar. 9	Oak log.	1,943	None found	104	102	235	3
5	Apr. 20	Linden log.	2,291	1	0	0	123	2
6	Feb. 9	Hickory tree.	2,139	1	0	0	867	0
7	Nov. 23	Boxelder tree.	1,872	1	0	0	216	7
8	Nov. 15	" "	1,246	1	0	0	196	2
9	Apr. 6	Linden log.	1,104	1	0	0	165	0
10	Apr. 20	" "	998	2	0	0	823	0
11	Apr. 13	" "	886	1	0	0	171	0
12	Apr. 6	Linden stump.	237	1	0	0	127	2
13	Apr. 13	Linden log.	167	1	0	0	74	0
14	Apr. 13	" "	139	1	0	0	74	0
15	Apr. 6	Linden stump.	122	1	0	0	106	0
16	Mar. 9	Oak log.	119	1	0	0	97	0

TABLE IV.

LARGE COLONIES OF *C. ferrugineus.*

No.	Date.	Situation.	No. Workers.	No. Wingless Queens.	No. Winged Queens.	No. Males.	No. Larvæ.	No. *X. cava.*
1	Dec. 28	Ash tree.	3,212	1	233	176	724	14
2	Apr. 13	Cherry log.	2,631	Not found	224	91	243	116
3	Apr. 20	Linden log.	2,214	1	131	97	322	51
4	Apr. 20	Oak log.	2,196	1	199	209	127	43
5	Mar. 23	" "	2,332	1	24	0	1,330	7
6	Apr. 6	Linden log.	327	1	0	470	75	22
7	Apr. 13	" "	143	1	0	0	87	7
8	May 4	" "	106	1	0	0	93	0

5. In the trees of the block in which I live are eleven colonies of *C. pennsylvanicus.* These colonies were observed almost daily after the first week of July until they ceased their activities with the approach of cold weather. Between July 18 and August 12 one of them, a very large colony, was seen daily to carry empty queen pupa cases from the nest. All the other ten colonies were watched carefully during this period, but only worker pupa cases were ever seen about them. The queens which were reared in

[1] The numbers in this colony are only estimated, as the ants were too active to permit of an accurate count.

the colony remained in the nest all winter, for I never saw any evidences of swarming, and two winged queens and one male were seen crawling about the nest on the evening of October 21.

6. On November 23 a friend who lives at Delavan, Illinois, discovered a large colony of *C. pennsylvanicus* which was living in a chest of small drawers which had been left undisturbed for three years in an old unused wood shed. This colony contained both males and winged queens.

From an inspection of Tables III. and IV. it will be seen, first, that with the exceptions of colony 6, Table IV., only the larger colonies contained the winged forms during the winter, and that all the largest colonies did contain them. In addition to this some of the larvæ of two of the large colonies which did not contain winged forms, viz., colonies 6 and 7, Table III., and which were kept in nests in the insectory after capture, proved to be male larvæ, and in both these colonies, moreover, the workers laid many eggs during the winter. As the queens of both colonies died soon after capture, the eggs that appeared in the nest must have been those of workers. It has been fairly well established that the eggs of workers usually develop into males, and hence one may be certain that these two colonies would have produced males during the summer of 1907 had they been left undisturbed; and since, as the tables show, winged forms of both sexes usually occur at the same time, it is quite probable that they would have produced queens.

Putting together the foregoing observations we may draw some more or less definite conclusions.

1. In two observed instances queens appeared in the adult form during the latter part of July and the first part of August, and in one of these instances these queens emerging at this time remained in the parental outdoor nest over winter.

2. One winged queen of *C. pennsylvanicus* was observed out of the parental nest on May 20; a colony of *C. ferrugineus* was seen in the act of swarming on June 12; and a colony of *C. pennsylvanicus* was reported to have swarmed during July.

3. All of the colonies of Tables I. and II. must have been established at least as early as July.

4. Nearly all the larger colonies were found to contain winged forms of both sexes during the winter.

5. None of the ten colonies of intermediate size which were observed closely during July and August were seen to carry queen pupa cases from the nest, and only one colony of this type represented in Tables III. and IV. contained winged forms during the winter.

These facts make reasonably evident the following conclusions:

First, that a colony does not produce winged forms until it is more than two years old.

Second, that a brood of winged forms is produced during one summer, remains in the parental nest over winter, and leaves for the marriage flight during a time ranging from May to July.

In regard to the number of years required for a colony to reach sufficient maturity to produce sexually perfect individuals I have the following data:

1. The two queens which reared their first young in artificial nests laid eggs at the rate of about two a day, during the regular season, and several others taken during the winter with small colonies have laid eggs at about the same rate part of the time since being brought into the insectory.

2. In the sixty colonies of Tables I. and II. the largest number of workers in any one colony is twenty-seven, and the largest number of larvæ is thirty-two.

3. In Tables III. and IV. we have the following rather distinct groups of colonies as regard size:

(*a*) Eight, with the number of workers ranging from one hundred and six to two hundred and thirty-seven, and the number of larvæ from seventy-four to one hundred and twenty-seven.

(*b*) Four, with the number of workers ranging from eight hundred and eighty-six to twelve hundred and forty-six, and the number of larvæ from one hundred and sixty-five to eight hundred and twenty-three. The colony with the largest number of larvæ, however, possessed two queens.

(*c*) Three, with the number of workers ranging from eighteen hundred and seventy-two to twenty-two hundred and ninety-one, and the number of larvæ from one hundred and twenty three to eight hundred and sixty-seven.

(*d*) Nine, with winged forms and with the number of workers ranging from nineteen hundred and forty-three to thirty-two hundred and twelve.

4. As I shall show in more detail later under polymorphism, the workers which a queen produces the first season are all of the very smallest size and, as the colony increases in size, larger and larger workers are produced until, in colonies of the size in group (*b*) above, a few of the largest size appear.

5. As I shall show later, under division of labor, these largest sized workers seem to take no part in the work of gathering food for the colony, but remain in the nest and seem to possess largely the instincts of queens.

6. Colonies 6 and 7, Table III. and colony 1, Table IV., were kept in artificial nests in the insectory after capture, and the workers laid eggs abundantly during the winter and a large number of these eggs developed into males.

7. Two smaller ones, 8 and 16, of Table III., were also kept in the insectory after capture, without queens, and were fed just the same as those mentioned above and no eggs were seen with either of these.

These data make reasonably evident the following conclusions:

1. Sexually perfect individuals are not produced until the colony consists of approximately two thousand workers, and they are produced by nearly all colonies of this size or larger.

2. From three to six years or longer are required for a colony to reach this size.

The fact that neither eggs nor pupæ are found in the nest during the winter, and that the larvæ are all very small, must mean that the proper feeding of the young larvæ and the egg laying cease several weeks before the temperature is too low for the process of incubation. This is supported by the fact that colony 1, Table III., which was taken on September 4, contained neither eggs nor pupæ, and only very small larvæ. The cessation of these two processes is probably caused by the workers and queens storing up food in their own bodies for the processes of metabolism during hibernation. If so, egg laying and the feeding of the youngest larvæ probably cease at about the same time, and the winter larvæ are hatched from the eggs which are in the nest when the queen stops laying. Hence we have in the number of winter larvæ an indefinite clue to the rate of egg laying in colonies of different sizes. The average number of larvæ in the

colonies of Tables I. and II. is 17.5 ; in those of group (*a*) it is 94 ; for the five queens of the four colonies of group (*b*) it is 271 ; in group (*c*) it is 402 ; and in group (*d*) it is 512. Doubtless a large number of the larvæ of the last two groups and possibly of one of the colonies of group (*b*) came from eggs laid by workers. This makes it reasonably evident that eggs are laid by the queen somewhat more rapidly after the first season than during that period when, as shown above, the rate is about two eggs a day. It is also evident that while the queen alone is laying eggs and it is quite probable that the workers do not lay eggs until just previous to the time of the production of winged forms the increase in numbers is slow enough to require several years to reach the two-thousand mark. Without arguing further a point based on uncertain evidence, I feel safe in believing that the colonies of Tables I. and II. are all one year old ; those of group (*a*) are two years old ; those of group (*b*) three years old ; of group (*c*) four years old ; and of group (*d*) five or more years old. Varying conditions may make the time of development of a colony vary, and so I feel sure that the time required for a colony to reach maturity is from three to six years.

As to the life of a colony after it reaches maturity I have the following data :

1. The average number of winged forms in the colonies of Tables III. and IV. which possessed them was 292. The queen larvæ especially must require a great deal more nourishment than worker larvæ, and after reaching maturity these forms remain in the nest for three or four months of warm weather and must be fed by the workers. Thus a large portion of the energy of the colony is consumed in rearing and feeding forms which annually leave it.

2. Colony 1, Table IV., contained a large number of winged forms when collected, and after these winged forms were removed from the colony, the workers laid a large number of eggs some, at least, of which developed into males. Thus it is probable that when colonies once begin to produce winged forms they continue to do so year after year. If this is true, the constant drain thus caused on the energies of the colony might cause it to degenerate in size, if the older workers should die faster than young ones

are produced. That in this way the life of a colony may come to a natural end, is supported by the following observations:

1. On June 12, 1906, I came upon a large oak tree which was hollow at the base, and was inhabited by a colony of *C. ferrugineus*. Fifty or more males were seen about the opening of the nest in a state of great excitement and the workers were dragging them back into the nest. About a month later I revisited the tree and not a single ant was seen about the place, although I watched it for about two hours. Twice afterwards I visited the tree with the same result.

2. November 3, 1906, I tore to pieces a large oak stump which was so badly decayed that I could break it to pieces with my hands. It was thoroughly riddled by the work of insects of various kinds, and showed plainly that it had recently been inhabited by a colony of *C. pennsylvanicus*, for besides the characteristic appearance of the galleries, I found fragments of workers' bodies, a few pieces of the wings of queens, and five live males and three large workers. Plainly a colony had recently moved from the place or had there reached the natural end of its life.

3. Colony 6, Table IV., with its 327 workers and 475 males, was very likely a degenerate colony which had about reached the end of its life. This colony was found in an old linden log which was so badly decayed and riddled by galleries which had evidently at different times been occupied by the colony, that it was just about to fall in pieces, and the whole scene presented every appearance of age. As is shown in Table VI., under "polymorphism," this colony contained a comparatively very large per cent. of the largest sized workers and a very small per cent. of the smallest sized workers, and this also is an indication that the colony had existed longer than the natural life-time of the small-sized workers which are produced in such a large proportion the first two years. The large-sized workers were produced later in the life of the colony and hence we might expect to have a larger per cent. of them in a degenerate colony.

Polymorphism.

The principal value of a knowledge of the complete life-history of a colony is, I believe, in the light which it will throw on the

problem of polymorphism among ants in general a problem on which much has been done and much written, but which I think, has not hitherto been examined from this standpoint.

The form of polymorphism we find here is what Eschreich in his "Die Ameise" calls incomplete polymorphism. That is, there is no distinct soldier-type, and there is a regular gradation in the size of the workers from the very largest to the smallest,

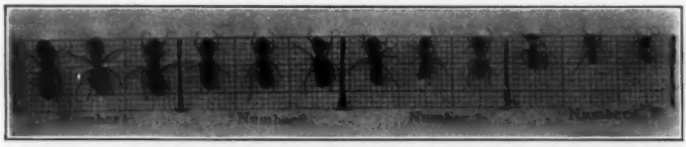


FIG. 3. Workers of *C. pennsylvanicus* arranged according to size and division of labor.

as shown in Fig. 3. I have grouped the twelve sizes shown in this figure into four subgroups to which I shall refer as Nos. 1, 2, 3 and 4, as indicated in the figure. This division into groups, I shall attempt to show later, is in harmony with an incomplete division of labor that exists among the workers.

The point revealed by a study of the life-history of a colony that I think is of importance is the fact that these different sizes of workers, and finally the winged forms, are produced at rather definite periods during the life of the colony. This is shown in Tables V. and VI.

The colonies represented in these tables were all killed in cyanide bottles and were then divided as accurately as possible into groups according to the four sizes represented in Fig. 3.

In all the colonies represented in Tables I. and II. every one of the workers was of size No. 4, and in the smaller colonies of Tables V. and VI., which, according to our previous conclusion, were two years old, this size still very largely predominated. In this latter group of colonies were found, however, a small number of size No. 3, and a still smaller number of No. 2, but not a single No. 1. From this point on, so far as the tables go, all sizes were found in all the colonies, and with the exception of colony 6, Table IV., not any perceptible increase is shown in the per cent. of the largest sized workers as the colony increased in

size, yet this per cent. varied within a narrow range for the different colonies. The fact that none of the largest size appeared in the one and two year old colonies cannot be merely accidental, for if all these colonies represented in the tables were taken together they would form a large colony, and yet not a single individual of size No. 1 would be found among them, while in the largest colony of Table V. there were 77 of this size.

TABLE V.

C. pennsylvanicus.

No. of Colony in Table III.	Workers Size No. 1.		Workers Size No. 2.		Workers Size No. 3.		Workers Size No. 4.		Total No. of Workers	No. of Larvæ.	No. Winged Queens.	No. Males.
	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.	No.	Per Cent.				
15	0	0	11	9.0	17	13.9	94	77	122	106	0	0
14	0	0	2	1.4	11	7.9	126	90.6	139	74	0	0
13	0	0	5	3.0	27	16.4	132	80.5	164	74	0	0
11	21	2.4	80	9.0	212	23.9	573	64.7	886	171	0	0
10	19	1.9	123	12.3	313	31.4	541	54.2	998	823	0	0
5	26	1.2	127	5.5	478	20.9	1,660	72.4	2,291	123	0	0
2	77	2.5	575	19.0	1,385	45.8	981	32.5	3,018	1,042	196	174
					C. ferrugineus.							
8	0	0	7	6.7	28	26.4	71	66.9	106	93	0	0
6	23	7.0	121	37.0	163	49.0	20	6.1	327	75	0	475
4	42	1.9	301	13.7	997	45.4	855	38.9	2,196	127	199	209
3	53	2.4	298	13.4	971	43.8	892	40.3	2,214	322	131	97
2	74	2.8	337	12.8	1,063	40.4	1,142	43.8	2,631	243	224	91

We may then consider it fairly well established that none of the largest workers are produced during the first two years of the colony's life, and that the sexually perfect forms are not produced until the colony is at least four or more years old. If it is true that the male forms usually arise from eggs laid by workers, we may add to the above that egg-laying workers do not appear until just preceding the production of winged queens. We then have a rather gradual increase in perfection from the smallest worker produced by the queen the season of her marriage flight to larger workers, then to egg-laying workers and finally to perfect winged females, and if the queen lives throughout this period, and it is altogether likely that she does, the eggs that produce these various forms are all laid by the same queen. An expla-

nation of this is not easy, yet I presume that I shall do no violence if I attempt one.

The most obvious external condition which may be responsible for this phenomenon is the food supply of the colony. During the first years the workers are few and the domestic duties are proportionally large. The permanent home must be established and there is consequently comparatively little time for food gathering, and probably in many cases relations are not readily established with a suitable herd of aphids, so that in all probability the larvæ of these years are scantily fed or are fed a less varied and less concentrated food than that given those that appear when the colony is more mature. During the later years the formicary is well established and there are larger and more powerful workers to make what extensions are necessary. The working force is increased proportionally more than the number of larvæ to be fed. These workers range over a wider field and collect not only more, but a greater variety of food. There can thus be little question that the larvæ of the large colony are better fed than are those of the small one, and since the better feeding is parallel with the production of more perfect forms, it seems only reasonable to believe that there is some relation between them. If it be asked, why do not all the workers of a given season develop into the same size or form, I think that I can say in reply that the food is not equally distributed. I have had numerous instances of a few of the larvæ developing much more rapidly than others in my artificial nests. The winter larvæ when taken were all practically of the same size, and many of them remained unchanged in size for several weeks after being brought into the insectory, while others, usually a small portion of the whole number, soon began to grow quite rapidly.

This leads us to the conclusion that the variations in form are ontogenetic in origin, that the fertilized eggs of the queen are all essentially alike when laid, and each capable of developing into a small worker or a winged queen. This conclusion is supported by a view proposed by Emery in a paper entitled "Die Entstehung und Ausbildung des Arbeiterstandes bei den Ameisen." I quote the following sentences of this paper from Wheeler's "Polymorphism of Ants" (*Bulletin of American Museum of Natural History*, Vol. XXIII., Article I.):

"The peculiarities in which the workers differ from the corresponding sexual forms are, therefore, not innate or blastogenic, but acquired, that is somatogenic. Nor are they transmitted as such, but in the form of a peculiarity of the germ plasm, that enables this substance to take different developmental paths during ontogeny."

Wheeler remarks in connection with this quotation that the view presented has never received the attention that it merits, and I trust that the data that I have brought out in connection with the life-history of a colony may serve to strengthen it appreciably. Wheeler has also elsewhere ("A Neglected Factor in Evolution," *Science*, N. S., Vol. XV., pp. 766–774) referred to the influence of the age and trophic status of the colony on the variability of the polymorphic ants.

Division of Labor.

Division of labor among the workers, like their polymorphism, is incomplete; and yet, in the one outdoor colony which I studied, very marked traces of it were seen. This colony, which I shall designate as colony A, lived in a large maple tree which stood on the border of a city block containing but three houses, the rest of the block being vacant, and allowed to grow up in weeds. One hundred and fifty feet away from the nest tree of this colony and just at the rear of one of the houses, stood a cottonwood tree, five or six years old; and near this was a clump of small boxelder trees. The cottonwood was infested with one species of aphis and the boxelders with another. The ants adopted these two "herds" of aphids as their main source of food, but showed a decided preference for those on the cottonwood. At the base of this tree they constructed a temporary chamber, by entering into a crack in the ground and carrying out the particles of earth as they do the particles of wood from their permanent home. After they had used this retreat for a time, I tore it open and, by means of glass plates, constructed a chamber for them somewhat like a Fielde nest, covering it with a piece of orange-colored glass through which I could easily observe what occurred beneath. This the ants readily accepted as equivalent to the one that they had constructed, and used it throughout the summer.

The first aphid appeared on the tree about May 1, 1906, and when I first noticed it a single ant was attending it. Gradually, as the colony of aphids increased in numbers and spread over the tree, the number of ants to be seen there also increased proportionally. My attention was very early called to the fact that, although some of the larger workers (size no. 2, Fig. 3) were often about the base of the tree, they never ascended to the aphids. The work of attending the aphids was performed entirely by workers of size no. 3. After the aphids became abundant I repeatedly saw ants of this size coming from the hole in the ground and ascending the tree to the aphids, and later returning. At the same time numerous workers of size no. 2 were coming from the nest and entering the hole in the ground, and others of this size were leaving this hole for the nest. On the evening of July 20, I made an observation which explained these actions. A no. 3 came down the tree and before entering the ground was accosted by a no. 2 and responded by giving up to her larger sister apparently all that she had gathered above. While their mandibles were interlocked in the process of transferring food the abdomen of the smaller one kept up a constant quivering, jerking motion, seemingly in an effort to regurgitate the last drop of food in her body. After this was over the smaller ones returned to the aphids, and the larger one entered the apartment in the ground as if not yet satisfied to return to the rest. This led me to construct the artificial chamber mentioned above and in this I have seen this process repeated many times. I find, however, that it is seldom that the no. 3 gives all the food that she has to offer to the first no. 2 that approaches her. During the daytime when only very few of the ants were active, a large number of the smaller workers were at rest in the quarters I had constructed for them and not much exchange of food was going on, but when I saw them here just after they had begun their evening's activities, or examined this chamber by means of a light, after dark, as many as two thirds of those in the chamber were paired off in the act of exchanging food. At these times, between the aphid tree and the nest was a caravan of workers going and coming, and these were very largely, though not wholly, of size no. 2. Those returning to the nest had their abdomens distended

until they appeared, at first glance, much larger than those traveling in the opposite direction. Thus, so far as this one colony is concerned, the food was gathered almost entirely by workers of these two intermediate sizes; the one, no. 3, so far as I have observed, without exception, first gathering the fluid from the aphids, and no. 2 principally transporting it to the nest, though aided by some of no. 3.

I have not observed other colonies sufficiently to determine whether this practice is general among them, though it surely cannot be universal for many colonies find their aphids on the same tree in which they live. This was a mature colony of large size and had probably perfected this division of labor gradually as the colony developed in conformity to the conditions that surrounded them.

The work of the two extreme sizes is more difficult to make out, for they are in some way the "house-keepers," that is, they are, during the daytime, at least, about the nest. The smaller ones, size no. 4, are often seen carrying the particles of wood and the empty pupa cases from the nest. Taking into account the immense mandibles of size no. 1, one might suppose that these are the true carpenter ants, and that it is their business to build additions to the formicary; but as has been shown in the tables above, they appear in the colony after it is pretty well established, and then occur in rather small numbers we must at least conclude that they are not the only carpenters in the colony. In general behavior they resemble very much the virgin queens. In the artificial nest they remain constantly in the dark room, and when the colored glass is removed they are among the first to seek shelter and the last to show fight. I have had since capture colony 6, Table III., in a nest which is connected with a feeding-room by means of four glass tubes each about three and a half feet long, three of them coiled and one straight, and I have never seen a no. 1, in the feeding-room, although there are fifty of them in the colony. The queen of this colony died soon after capture, and yet many eggs have been laid, I consequently feel quite certain that the no. 1's are principally egg-layers, and it is probable that along with the development of the ovaries some of the instincts of the queen also appear, and a corresponding lack of certain other instincts possessed by the common workers.

Food.

The food of these ants consists principally of the so-called "honey dew," of the aphids, this being supplemented by insect food, and occasionally by plant juices. While they may prefer some species of aphids to others, they are not limited to any one, and seem to be able to make use of all species that infest the aerial parts of plants. I have seen them attending aphids on burdock, on wild lettuce, and on spruce trees, as well as on plants whose sap has a more pleasant taste to us. The aphids are not domesticated as are those of some other species of ants, and I have found no aphids or aphis eggs in any of the nests that I have opened. In October, when the aphids on the cottonwood tree above mentioned were laying eggs, I repeatedly collected leaves to which eggs were attached, and placed them near the base of the nest tree and at various other places in the direct path of the ants, but although I repeatedly saw the ants pass directly over the eggs, they paid no attention to them.

So far as my observations go, their insect food is never taken alive. These ants seem to live peaceably with all creatures so long as the portals of their formicary are not crossed, and they give free admission here to a good many special guests. They do not even attempt to monopolize the herd of aphids which they attend, but seem to admit the equal claim of other species of ants. When a dead insect is found by them, a number of workers gather around it and suck out its fluids, which they then carry to the nest, leaving the dry, chitinous skeleton behind. I have noticed, however, that they nearly always carry the hard chitinous head of an insect into the nest, and I have often wondered why this is done. In the actions of ants living in my artificial nests I think that I have found explanation. After feeding these colonies a number of white grubs, I have noticed that the head is always carried from the feeding-room into the nest. Here it remains for a few days, and then the empty shell, which has been divested of the last particle of soft tissue, is thrown upon the rubbish heap. It may be that in the head is a choice bit of food, possibly the brain of the larva, which is served directly to some special member of the household, or it may be

that some special skill, not possessed by the foraging worker, is required to extract it.

I have frequently seen individuals of *C. pennsylvanicus* feeding on an apple, and on one occasion saw them extract the juice from a large stalk of pie-plant. This material was available to them for some time during the season, but they helped themselves to it only once. I once saw a colony which lived in the trunk of a large ash tree feeding on the pulp of a water-spout of the tree. They had removed almost every particle of material from within the bark for a distance of about a foot, so weakening the sprout that it had bent over. This had been done within a short time, for the leaves above the injury, although wilted, were still green, and a few of the ants were yet working on it when I saw them.

These ants seem to possess great power of husbanding the nutriment within their own bodies. I have kept colony 1, Table IV., in the insectory since January, giving them no food but sugar and water, and yet they have successfully brought to maturity all or nearly all their larvæ, their workers have laid many eggs, and the colony is now, May 10, to all appearances, as healthy as any under my care. The proteid food required for the feeding of the larvæ and for maturing the eggs must have been in store in some form in the bodies of the workers. I have also noticed, with respect to the colonies which I have collected since the few warm days we had in March, that many of them are much larger than any I saw in outdoor nests previous to that time. Very few of these ants have even yet, May 10, been seen out of the nest, and the food upon which the larvæ have grown must have been a surplus of that stored for the purposes of respiration during the winter. I have two colonies, viz., 16 and 12, of Table III., to which I have given no food since April 6. Colony 16 had been given the usual indoor fare since capture up to the time mentioned above, and colony 12 was captured on the day the experiment began. Both colonies are now, May 10, apparently as healthy as any others that I have in confinement.

This faculty adapts them admirably to the conditions of their life, for gathering their food as they do, and being unable to store it otherwise than in their bodies, there is likely to be consider-

able variation in its character, and considerable fluctuation in its amount.

Relations to Light and Color.

In endeavoring to work out the relations of these ants to light and color I have resorted to experiments modeled after those made by Fielde, Lubbock and others, on other species of ants. I have, however, used slightly different apparatus from that used by either of these investigators. In the first place I constructed a nest of the Fielde type which was twenty-four inches long and nine and one half inches wide, and which contained a hall-way one and one half inches wide, running longitudinally through the center, with six rooms, each 4 by 4 inches, on either side of the hall-way. The outer walls of the nest were bound by black binding paper and the walls between the rooms were made of two pieces of glass with a strip of black paper between them, so that all the walls of each room were perfectly darkened and no light could enter the rooms except through the glass plates placed over them and through the small pieces of glass tubing which formed the entrances from the hall-way. The nest was connected with a feeding-room by means of a piece of glass tubing which led from one end of the hall-way. The hall-way was covered with a strip of clear glass, and as covers for the rooms I used glass plates of the following descriptions:

1. A deep red glass which transmitted only the red rays of the spectrum.

2. A brownish orange glass which transmitted all of the red end of the spectrum including a large part of the green.

3. A green glass which transmitted all of the green rays and a small part of the red.

4. A deep blue glass which transmitted all of the blue end of the spectrum, including a very little of the green.

5. An indigo-blue glass transmitting all colors of the spectrum to some extent, but showing narrow absorption bands in the red and green.

I also used cells containing carbon disulphide to shut out the ultraviolet rays.

With this apparatus I performed the following experiments:

Experiment I.

January 1, 1907. — On one side of the hall glasses were placed on the rooms in the following order: red, orange, green, indigo, blue, clear; and on the other side in the reverse order, so that the two red glasses were on diagonally opposite corners of the nest. A colony of *C. pennsylvanicus* was introduced into the feeding-room. This colony was just large enough to fill comfortably two of the rooms, and too large to get into one.

January 2. — All ants were settled in the two rooms covered by red glass. The red plates were now exchanged with the two clear plates, and on January 3, all ants were again collected under the red glass. These red plates were now removed from the nest and were replaced by plates of clear glass. On January 4, about one third of the colony were in one room under green glass, another third were under orange, and the remaining third stayed where they were under clear glass for four days, finally joining their companions under the green and orange respectively on January 8. These glasses were now exchanged with the two indigoes, and the ants remained unsettled for a whole week of dark, cloudy weather, as many of them remaining under clear glass as under any other. On January 15, I placed double thicknesses of orange glass over two of the rooms, and on January 18 all ants were collected in these two rooms. I now removed the orange glasses from the nest, replacing them with clear glass, and on January 19, after a few hours of bright sunshine, the ants were all collected under the two green glasses. These were then exchanged with clear glass and the ants were again unsettled for a period of six days, when a bright day caused them to settle under the green glasses on January 25. These glasses were now removed from the nest and replaced by clear glass. The nest was then left for twelve days in this condition with only the blue, indigo and clear glass over the rooms, and although there were several bright days during the time, the ants never settled in any one room, but seemed to be endeavoring to escape. On February 6 I replaced two of the clear plates with the two green plates, and on February 8 all ants were collected in these rooms. I then placed the orange plates back on the nest, but no ants collected under them. On February 15 I

placed the red plates back on the nest and the following day about twenty ants were collected under one of them, but not all of the ants removed to the red glass, however, until February 22. The glasses were now arranged as at the beginning of the experiment and the carbon disulphide cells were placed over the two clear plates. The experiment now proceeded as before, with no essential difference in results, until the red, orange and green glasses had been removed from the nest. The green plates were removed on March 8, and two days later all ants were collected under the two disulphide cells. I then returned the green plates to the nest, and the following day ten ants were in the room under one of them. The number that left the disulphide cells for the green gradually grew until, on March 17, all had done so.

Experiment II.

This colony was now removed from the nest and, after the latter was thoroughly cleaned, another colony of *C. pennsylvanicus* containing a large number of winged queens and males was introduced. The glass plates were again arranged as at the beginning of the experiment and the disulphide cells were placed over the clear plates. The colony would have filled about three of the rooms, but they scattered out and occupied eight of them, omitting entirely, however, the two indigoes and the two blues. The nest was left as first arranged from March 20 to May 12, and some ants were seen at all times in each of the eight rooms in which they first settled, except a few days while the disulphide cells were removed. During all this time only occasional stragglers were ever seen under the blue or the indigo plates.

Experiment III.

A number of the queens of the colony used in experiment II. were removed for other experiments and the colony was reduced in size until it could easily occupy two of the rooms, and then, on May 12, the nest was taken into a room which admitted no light from the outside and which was supplied with an arc light of four hundred and eighty candle power. The nest was placed about three feet below the arc light, and a little to one side so as to avoid the shadows of the lamp. The glass plates were

arranged as in experiment I., and the disulphide cells were placed over the clear plates. The ants immediately collected under the red plates after the arc light was turned on. These plates were then exchanged with the two blue plates, and twenty-five minutes later the ants were again under the red plates. These plates were then replaced by clear plates, and thirty minutes later all ants were collected under the two orange and one of the green plates. The orange plates were again exchanged with the blue, and in twenty-five minutes the ants were under the two green and one of the orange plates. The green plates were then removed from the nest and thirty-five minutes later all ants were under the two orange plates. These were then removed from the nest and forty minutes later all ants were under the two disulphide cells. The two disulphide cells were now placed over the blue plates and in twenty-five minutes all ants were collected under them. Next the cells were moved to the indigo plates and in thirty-five minutes all ants were again under them. The cells were then placed back on the clear plates and in thirty-five minutes the ants were again under them. The red plates were then placed back on the nest, and two hours later some of the ants were still under the disulphide cells, although most of them had moved to the red plates.

These results indicate plainly that, when forced to choose between light of different wave-lengths, these ants have a decided preference for the red or longer rays and a decided dislike for the ultraviolet rays. The last part of experiment III. also indicates that they prefer the red rays to the blue and violet rays. In these respects these ants seem to agree perfectly with the ones which Lubbock experimented upon ("Ants, Bees and Wasps," pp. 211 to 217) and also with those of Miss Fielde ("Notes on An Ant," Philadelphia Academy of Science, Vol. 54, pp. 614 to 625).

Experiment IV.

Twenty-five workers of *C. ferrugineus* were cooled until they were inactive and then their eyes were carefully painted with a mixture of liquid glue and lamp black. They were then placed in a Fielde nest consisting of two rooms, four by four inches, which were joined by a narrow passage-way. One room was

covered with a red glass plate, such as described in experiment I., and the other with clear glass. The nest was now exposed to the arc light. At the beginning, the ants were all placed under the red glass, and after the light was turned on, the glass plates were changed about. The ants showed some uneasiness, and yet remained under the clear glass for two hours, seeming to be utterly ignorant of the fact that they were exposed to bright light. As a check on this experiment a similar number of workers from the same colony were placed in a similar nest, but their eyes were not painted. By changing the glass plates I was able to cause them to move from one room to the other fifteen times in thirty-five minutes. This makes it evident that the effects observed are due to the light as perceived through the eyes.

Experiment V.

Twenty workers and five queens of *C. pennsylvanicus* were placed in a hollow cylinder formed by rolling up a strip of fine wire screen and stopping the ends with corks. A centigrade thermometer was thrust through a hole in one of the corks so that the bulb was in the center of the cylinder. The cylinder was now held for an hour about four inches from the arc light. In this position the thermometer registered about 40° C. The ants were exceedingly active all the time, and showed no ill effects afterwards. I have found by other experiments that these ants are able to endure a temperature of 40° C. indefinitely without serious effects, but that they are very suddenly killed when the temperature reaches 46° C.

These results indicate that these ants are adapted to withstand very intense light which is rich in ultraviolet rays, and so, evidently, their nocturnal habits are not a result of necessity, but of simple preference.

There are always some of the workers busy during the daytime of the active season, but the vast majority remain quietly in the nest, and then, a few minutes after sunset, the whole colony seems to awake and the night's labors begin.

August 12, 1906, I observed the outdoor colony *A*. I began counting the ants which left the temporary nest at the base of the aphid tree to collect food from the aphids, just as the sun

disappeared from sight. During the first eighteen minutes I counted only twenty-two ants, and then, as if by a sudden signal, the procession began to move, and during the following eighteen minutes I counted five hundred and twenty-two ants, and about this time the supply in the nest below seemed to be almost exhausted. A little before I stopped counting, large numbers of the larger workers began to arrive from the nest tree and to enter the temporary nest at the base of the tree. I have observed this colony repeatedly at different times during the night, and as late as two o'clock in the morning, and have always found them very active.

Architecture and Economic Relations.

McCook describes in detail the architecture of a colony of carpenter ants which he found inhabiting a corner beam of an old mill. ("A Guild of Carpenter Ants," *Harper's Monthly*, July, 1906.) In the same article he discusses serious injuries to forestry and lumber interest which have been reported to have been done by the carpenter ants, and he also reports railroad accidents which were thought to have been caused by carpenter ants weakening the timbers of bridges.

Dr. E. P. Felt, state entomologist of New York, also accuses the large, black carpenter ants of doing much injury to forests. ("Insects Affecting Forest Trees," Seventh Report, New York State Commission of Forest, Fish and Game, p. 522.) In this

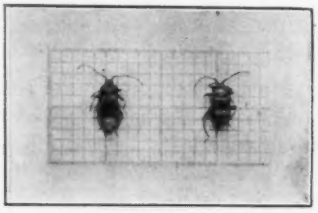
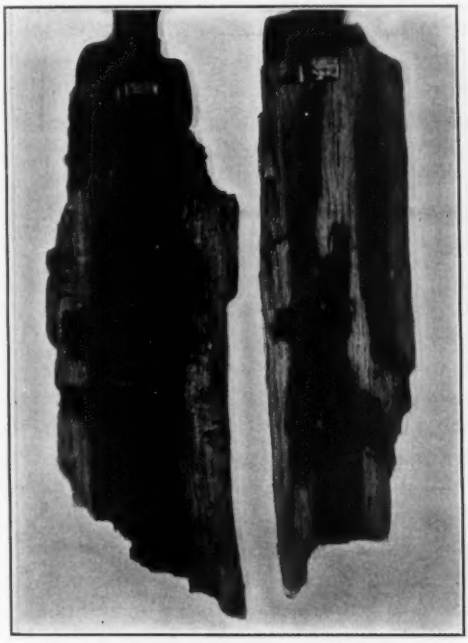


Fig. 4. Two specimens of *Xenodusa cava*.

article he shows in cuts the very different styles of architecture followed by the ants in elm and in balsam and supposes that the difference is due to the different structures of the two woods.

The observations which I have made while collecting the colonies represented in the tables of this paper lead me to believe

that the carpenter ants are guiltless of much that they are charged with and also that they may scarcely be credited with having a distinctive architecture of their own. In solid wood they follow the burrows of wood-boring larvæ almost exclusively making very slight changes as long as the wood is solid. In Fig. 4 is shown a piece of wood taken from a large ash tree which was hollow for a few feet at the base, and which was inhabited by a large colony of *C. ferrugineus* and numerous wood-



5 6

FIG. 5. View of a gallery of *C. ferrugineus* as seen in a radial section of decayed oak.

FIG. 6. Same as Fig. 1, as seen in tangential section.

boring larvæ. Both the ants and the wood-bores had entered from the inside and were working outward toward the living parts of the trunk. The ants were living entirely in the unaltered galleries of the larvæ. All that they had done was to clean out

the voided wood that the larvæ had left in the burrows. As the wood gradually decays and in cases where the ants follow larvæ that bore in decayed wood they do enlarge the galleries and shape them into chambers which are more or less characteristic, but even here the style of the architecture is determined largely by structure of the wood and the instinct of the particular larva followed. The influence of the structure of the wood is shown in Figs. 5 and 6. Fig. 5 shows a radial section of decayed oak in which the more durable medulary rays formed a limit to the gallery in one direction and in Fig. 6 is shown a tangential section of the same wood in which the dense summer wood of an annual ring forms the limit in the other direction. Many observations similar to these have convinced me that these ants do not build their own galleries in solid wood. They either follow the wood-bores or work in badly decayed wood and if this conclusion is true their economic importance must be extremely slight.

Guests and Parasites.

Probably the most distinguished and interesting guests which have been found with ant colonies are certain Lomechusini, several species of which are very common guests with the ants of continental Europe. This family is represented in North America by the single genus *Xenodusa*, and the best known representative of the genus is *X. cava*, Fig. 7. Wheeler, in a review of the observations made on this beetle ("Polymorphism of Ants," *Bulletin American Museum of Natural History*, Vol. XXIII., pp. 35–40), shows that, so far as reported, only five persons, viz.: Leconte, Blanchard, Muckermann, Schwarz and himself, have ever seen it and each of these only rarely. Schwarz found it with *C. pennsylvanicus*, and Blanchard with a colony of large black ants which were probably of the same species. No one has before seen it with *C. ferrugineus*. By referring to Tables III. and IV. of this paper, it will be seen that I have found it to be quite abundant in this region, and by a comparison of these two tables, it will be seen that the beetle seems to prefer *C. ferrugineus* as a winter host. Wassmann and Wheeler are of the opinion that the beetle simply hibernates with these larger ants and then in the spring migrates to the nests of some smaller ants

as a summer host. In fact Wheeler found, July 1, 1905, six larvæ of *X. cava* in a nest of *Formica incerta*, and since the European relatives of the beetle are found mainly with this genus

FIG. 7. Galleries in solid ash wood which were inhabited by *C. ferrugineus*.

of ants, it seems quite probable that the summer host of *X. cava* may be some one or more of the *Formicas*. During the month of June, 1907, it was my hope to discover the summer host and I accordingly opened a good many nests of *Formica* in the vicinity of the woods in which I found the beetle most abundant, but was unable to find a trace of either adult beetle or of larvæ. I did, however, find adult beetles in the nests of *C. ferrugineus* at differ-

ent times during the month. On June 10, I opened two nests seeing five beetles in one and three in the other; on June 20, I found four beetles in a nest of these ants and on June 30, I opened a nest and saw two of the beetles. These may have been a few stragglers which had not migrated because of some disability yet I could not see any signs of imperfection in them. Since Wheeler found larvæ on July 1, it seems quite probable that at least some of the beetles spend the summer also with the winter host.

The beetles are always royally received into the nests of the two varieties of ants that I have been studying. I have kept them all winter in artificial nests with the ants, have transferred them from one colony to another and have placed them in the nest with colonies which had none in their out-door nests, and they were always received and cared for. I have repeatedly seen the ants licking them and feeding them, and when the beetles strayed out to the feeding-room of the nest the ants would pick them up and carry them back to the nest.

Another guest or parasite which I have found abundantly with the colonies of *C. pennsylvanicus* is a small red mite, which, according to Nathan Banks, to whom I sent some mounted specimens, is an undescribed species of *Uropoda*. I have not been able to determine whether these are parasitic or merely attached to the ants for the purpose of transportation, but am inclined to think the former, as was also Mr. Banks. They were always attached to the ants at the joints of the legs, or on the underside of the joint between the head and thorax and were probably extracting their nourishment through the thin nonchitinous membranes of these regions. It seems a little peculiar that the ants permitted them to stay there, for evidently they could easily have removed them. It is possible, however, that the mites secured this attachment after the ants became inactive from the cold, for I collected them all during the winter and noticed that the mites disappeared within two or three days after being brought into the insectory.

Besides these I have found numerous other insects which live either in the nest or in very close proximity to it and seem to live peaceably with the ants. Among these are various staphy-

linid beetles, several species of Blattidæ, and the adults of the wood-boring larvæ. Other ants are also often very close neighbors of the species I have studied. I have found two colonies of small ants in chambers which were connected on all sides with those occupied by the larger ants. One of these was a colony of *Myrmecina americana*, which I found with a colony of *C. ferrugineus*, and the other a colony of *Monomorum pharaonis*, which I found with a colony of *C. pennsylvanicus*. The insectory is badly infested with this latter species and they have helped themselves liberally to the food that I have given my ants, and have especially thrived on the dead ants which my colonies threw upon their rubbish heaps. These little ants are not noticed by the larger ones, and doubtless in the natural nests they subsist largely by picking up the "crumbs" about the formicaries of the larger species.

Instincts and Intelligence.

The many remarkable feats performed by ants in the round of their daily life have led observers to form various conclusions as to the parts played by instincts and intelligence respectively, in controlling their movements. Lubbock, on the one hand, concludes that ants rank next to man in the degree of intelligence possessed ("Ants, Bees and Wasps," p. 1), and on the other hand, Wassmann endeavors to show that they are absolutely void of pure intelligence ("Psychology of Ants").

On this subject I have made the following observations and experiments:

Observation 1.— On the evening of August 1, 1906, I planned to count the number of workers of outdoor colony *A*, which passed a certain point between the nest and the aphid tree, in a given time. In order to do so I cleared away the weeds so that I might see them more easily. I soon noticed, however, that ants were collecting on both sides of the place where I had disturbed the path, and they refused to cross the disturbed area. Considerable excitement seemed to prevail on both sides, and the number kept increasing, especially on the side toward the nest. Some of these returned to the nest and some of the others returned to the aphid tree. Finally, after about thirty minutes, when approximately one hundred ants were assembled on the

side toward the nest, this group began to advance slowly into the disturbed territory. After much retracing of steps they succeeded in about three minutes in crossing this area, which was only about one foot in diameter, and they then hurried on their way unhesitatingly.

Observation 2.— About fifty feet of the path of this colony from the nest to the aphid tree lay through a dense weed patch. Early in the summer I had smoothed out with a hoe a narrow path in a fairly direct line from the nest to the aphid tree, and by repeatedly passing along this path myself, I kept it worn smooth all summer. I repeatedly observed that the ant caravan used this path only at points where it happened to coincide with a perfectly direct line between the point at which they entered the weed patch and the point at which they emerged from it.

On the afternoon of August 4, 1906, some men came with a plow and tried to plow the weeds under. The weeds were three or four feet high, and when the plowing was finished the lot seemed to me an impassable barrier to the movement of the ants. That evening, as the ants began to pour forth from the nest for their night's work, great excitement ensued when they reached the edge of the plowed ground, which was about five feet from the nest. Soon a space about one foot wide and reaching from the nest to the plowed ground was literally black with ants, all running back and forth and behaving very much as people do in case of a fire in a city. After about twenty minutes of confusion the vanguard began to advance slowly into the plowed ground, and in just two hours they had reached the other side. During all this time about one hundred and fifty ants which had started from the aphid tree to the nest were collected near the border of the plowed ground, but not one of them ventured as much as an inch into it. The next day and evening they were traveling back and forth just as if nothing had happened, and the path that they followed was so straight that when I stretched a line across between the two points of entrance no ant was seen to be more than five inches to either side of the line anywhere along the course. A decided curve was made in the path, however, after reaching smooth ground on the side toward the aphid tree.

Experiment I.

December 24, 1906, I connected a Fielde nest containing a colony of *C. pennsylvanicus* with a feeding-room by means of a series of five glass tubes, each about six inches long. Five days later, when the ants had become accustomed to these tubes, I turned one of the sections of the tube end for end and ants passed through in both directions without seeming to notice the change. Next I removed one of the sections and replaced it with a new piece of tubing of the same size. At this time three workers were in the feeding-room, and soon one of them started to go to the nest. She went hurrying along the tube until she came to the new section, when she suddenly stopped and began feeling cautiously about. She then made several trips to the feeding-room and back to the new section, but did not venture a full length into it. While she was continuing in this way another worker came from the nest and she too came to a sudden stop on reaching the new section of the tube. She examined it carefully and then, without returning to the nest, proceeded cautiously through it. Here she met her friend who had formerly discovered the change, and after they had exchanged antennal greetings, the two returned to the nest. This experiment was repeated a number of times and always with the same results, that is, those ants which were in the feeding-room always refused to cross the new section until they had met some friends directly from the nest, while those coming directly from the nest always crossed the new section, at least, after making one trip back to the nest.

Experiment II.

The same nest as above was connected with the feeding-room by means of a glass tube four feet long which had been bent at the center to form an angle of about 110°. The ends were bent in the opposite direction, so that by slipping the ends of this tube over the smaller tubes which led from the nest and feeding-room respectively, and by rotating the larger tube about the smaller ones as axes, I could vary the elevation over which the ants must travel in passing from nest to feeding-room. The tube was allowed to lie flat on the table usually, and at intervals I would raise it up so as to cause a steep incline. When this was done

ants which were in the feeding-room would not hesitate to pass through it, but they would invariably try to walk on the side which was lowermost when in the usual position.

Experiment III.

I have repeatedly taken a stick and made a narrow mark in the earth across the path of the ants from the nest to the aphid tree and have observed that only very few of the ants going from the nest to the aphid tree seem to notice it, while nearly all those going in the opposite direction would stop and examine the mark carefully for some time, and some would return to the aphid tree rather than cross the mark.

These experiments show clearly that the ants behave differently when traveling from the nest than when returning to it. They are seemingly willing to venture into new territory when traveling away from the nest, because of something akin to a consciousness that they can at any time retrace their steps and find the nest, while when traveling toward the nest the link is broken when the surface of the earth is disturbed across their path. This may be either because the continuity of the outgoing trail is destroyed, or because the appearance of things with which they are familiar is altered.

Observation 2 seems to indicate quite strongly that these ants possess a sense of direction and an unusual power of using it under unfavorable circumstances. The rough ground all matted with weeds must have appeared to them much as a mountain region over which a cyclone had torn the forest to shreds would appear to us, and yet they made a straight path across it in the darkness. Their determination to cross this hazardous region at once seems also to imply that had some realization of the interests at stake and some memory of the direction in which the goal lay.

Experiment IV.

A Fielde nest containing a large colony of *C. pennsylvanicus* was connected with a feeding-room by means of a system of four glass tubes, each one half inch in diameter. One of these tubes was straight, another was bent into a vertical loop, another into a horizontal loop, and the fourth was arched so as to form a steep

incline. All four were brought together at the ends into triangular-shaped vestibules. A single tube led from one of these to the nest, and one from the other to the feeding-room. After this apparatus had been set up for forty-eight hours and the ants had become somewhat accustomed to the system of tubes, I placed in the feeding-room about two hundred larvæ which had been taken from the nest two hours before. Two small workers were in the tubes when the larvæ were placed in the feeding-room. They were apparently lost, for they divided their time between remaining perfectly motionless as if trying to gain their bearings, cleaning their antennæ, and running frantically about in the tubes. After about twenty minutes of such conduct, one of them entered the feeding-room and discovered the larvæ. She examined them carefully with her antennæ and then, with more excitement than before, renewed her search for the nest. She ran wildly about the system of tubes and the feeding-room for twenty-two minutes, and then found her way from the vestibule to the nest. On reaching the nest she ran against five of her companions very much as ants do when they first discover a stranger in the nest, and she then returned directly to the larvæ, passing through the straight tube. The five friends which had been greeted in this peculiar way turned around a time or two and then followed their informant immediately into the tubes, all passing into the straight tube, and three of them going directly to the larvæ in the feeding-room. The other two seemed to lose the trail in the second vestibule, and began running about the tubes. Each of the four who reached the larvæ began carrying them into the straight tube, and after making three trips from the larvæ to this tube, the original discoverer of the larvæ returned to the nest and, by the same behavior as before, succeeded in bringing three others to the scene of activity. Before all the larvæ were removed from the feeding-room five ants had returned to the nest for help and each time secured it. Thirty ants were in this way called into service, yet not an ant left the nest which had not been greeted in this peculiar way. After all the larvæ had been carried into the straight tube, the ants began carrying them into the nest, and as the larvæ arrived in the nest other ants joined in the work, so that the tubes were soon alive with ants.

This experiment was repeated a number of times and each trial gave unmistakable evidence that the ant which discovered the larvæ in some way conveyed the intelligence to others. I have also had similar evidence of communication when I placed the carcass of a white grub in the feeding-room of a colony that had been deprived of animal food for some time. In the succeeding trials of this experiment, however, it never happened again that all the larvæ were returned to the nest through the straight tube. They were as often carried over the vertical loop as through any other tube, and this happened just as frequently after the ants had used the tubes for three or four months as at first. The ants always work as if in great haste to get the larvæ back to the nest, and it seems that if they had had any discretion whatever, they would have chosen the shorter and less difficult route. In one instance, however, I watched an ant while making six trips from a cluster of larvæ which had been carried into the straight tube. Three of the six times, when returning to the larvæ, she entered the arched tube from the vestibule and proceeded until she came to the incline and then each time turned about and found the straight tube and the larvæ. She seemed to remember that the larvæ were in the straight tube and so knew that she was in the wrong tube when she came to the incline. She also seemed not to be tracking herself as she returned to the larvæ.

Experiment V.

Three islands were formed by inverting two-inch Petri dishes in four-inch ones and filling the larger ones with water. One of these islands, which I will designate as *A*, was connected with the feed-room of the apparatus used in experiment IV., by means of a glass tube which was bent in such a way as to be partly immersed in water in a Petri dish. This made it impossible for the ants to crawl back to the feeding-room over the tube and escape to the table. The other islands, which I will designate as *B* and *C* respectively, were connected with island *A* by means of bridges of cardboard eight inches long and one half inch wide. *B* was placed in a direct line with the tube leading to *A*, and *C* was placed at right angles to the tube, opposite *A*.

Larvæ taken from the colony in the nest were placed on *B* and

the bridge to *C* was removed. After about one hour a small worker discovered the tube and cautiously followed it to *A*, and then passed over the bridge to *B*, where she discovered the larvæ. After examining them carefully she started to find her way back to the nest. After passing through the feeding-room and into the vestibule she entered the tube with the horizontal loop and went as far as the loop and then turned about to the vestibule and thence went through the arched tube and to the nest. In the nest she saluted four of her friends in the manner described in experiment IV., and returned to the larvæ followed closely by the four friends saluted and no others. In going to the larvæ she passed through the arched tube by which she had returned to the nest, but instead of following the diagonal path by which she had previously crossed the feeding-room, she followed around the sides of this room until she came to the entrance to the tube leading to island *A*. Each of the five ants on arriving at *B* picked up a cluster of larvæ, carried them to the place where the tube leading from *A* passed under the toweling of the feeding-room, dropped them there as in a place of temporary safety, and returned for more. While the ants were in the tube with the third load, I moved the bridge from *B* to *C* and placed a new bridge leading to *B*. The first and second ant to come back to *A* passed over the new bridge to *B*, but the other three, after turning around once or twice on *A*, passed down the old bridge to *C*, retracing their steps, however, a few times before finally reaching it. They then returned to *A* and finally found the larvæ again. I now allowed them to pass over this bridge to *B* about eight times and then again moved this bridge to *C*, taking away the one there already, and placing a new bridge from *A* to *B*. This time all five of the ants passed directly over the new bridge to *B*, and I could not see that any of them detected the change. After all the larvæ had been removed from the island to the tube, three of the five ants began carrying them to the nest while the other two returned to the nest empty-handed for help, and my observations ended in the confusion that soon followed.

This experiment was repeated a number of times; and while there were a few variations in results, those recorded in detail

above are typical. The principal variations are the following: Sometimes instead of waiting for an ant to find the larvæ, I took one directly from the nest and put her with them. When this was done the ant invariably picked up a cluster of larvæ and sought to find a temporary place of safety for them. In this she sometimes succeeded, and at other times she brought the larvæ back to the others on the island and then found the way home before again touching them. In no instance, however, did an ant which had found the larvæ herself attempt to carry them away until she had made a trip to the nest.

In one trial the ant which found the larvæ called on eight of her friends to help before she started to lead them to the larvæ. While she was doing this two of the first she had saluted started for the larvæ ahead of her and went directly to them, following exactly the route over which their informant had returned to the nest.

If I changed the bridge from *B* to *C* and replaced it with a new one while the first discoverer of the larvæ was on her first trip to the nest, those that followed her back invariably went to *C* and, not finding the larvæ there, they often returned to the nest and seemed to give up the search as if they had been falsely advised. But, although the original discoverer sometimes also followed her old trail to *C*, I never knew one which had really seen the larvæ to give up searching until she found them again.

I think that these experiments and observations fully warrant the following conclusions:

1. These ants have some means of inter-communication. A. Bethe has endeavored to show that all so-called communication among ants may be explained by odors carried by the informants and perceived by those saluted (Dürfen wir den Ameisen und Bienen Psychische Qualitaten zurchreiben). But in this case I do not see how the ants saluted could have known that the odor of the larvæ which the informant may have borne was not received from the larvæ in the nest. It is perhaps possible that the larvæ removed from the nest give off some special odor which is a signal of distress and which may be conveyed to the nest by the informant, but I think this far less probable than the other explanation.

2. These ants are capable of tracking themselves and others of the colony, but they are not capable of distinguishing the direction in which the trail was first laid down.

3. They do not depend entirely on following trails in finding their way, but are guided often by a kind of memory of the location of things, and probably depend as a last resort on a sense of direction.

4. They ordinarily pay very little attention to trails when traveling from the nest.

5. They give no evidence, in these operations, of anything akin to reason.

BIBLIOGRAPHY.

This bibliography contains only those articles referred to in the text of the paper and those containing accounts of experiments which I have used as models in my work.

Bethe, Albrecht

'98 Dürfen wir den Ameisen und Bienen psychische Qualitäten zuschreiben. Sonder-Abdruck aus dem Archiv für die gesammte Physiologie, 1898.

Emery

'94 Die Entstehung und Ausbildung des Arbeiterstandes bei den Ameisen. Biol. Centralbl., XIV., 1894.

Escherich, Dr. K.

'06 Die Ameise. Friedrich Veeweg und Sohn, 1906.

Felt, Dr. E. P.

Insects Affecting Forest Trees. Seventh Report of Forest, Fish and Game Commission, State of New York, p. 522.

Fielde, Adele M.

1. Portable Ant Nests. Biological Bulletin, Vol. 2, No. 2.
2. Study of an Ant. Proceedings of Philadelphia Academy of Natural Sciences, Vol. 53, p. 425.
3. Further Study of an Ant. Proc. Phila. Acad. Nat. Sc., Vol. 53, p. 521.
4. Notes on an Ant. Proc. Phila. Acad. Nat. Sc., Vol. 54, p. 599.

Lubbock, Sir John

'01 Ants, Bees, and Wasps. D. Appleton & Co., 1901.

McCook, Dr. H. C.

'06 A Guild of Carpenter-Ants. Harper's Monthly Magazine, July, 1906, p. 293.

Wassmann, Rev. E.

'05 Psychology of Ants and of Higher Animals. B. Herder, 1905.

Wheeler, William M.

The Polymorphism of Ants, with an Account of Some Singular Abnormalities Due to Parasitism. Bulletin American Museum of Natural History, Vol. XXIII., p. 1.

'02 A Neglected Factor in Evolution. Science, N. S., Vol. XV., No. 385, pp. 766–774, 1902.